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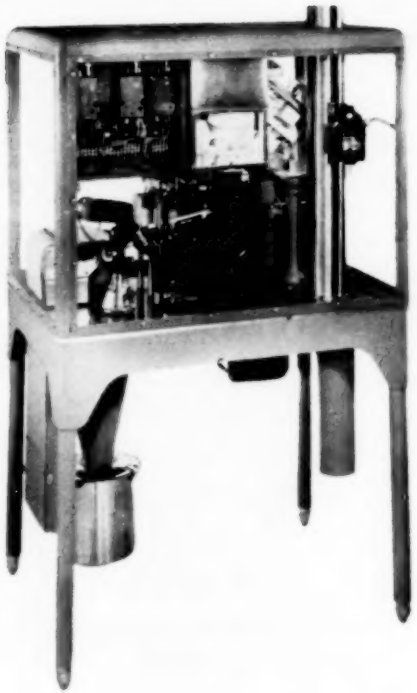

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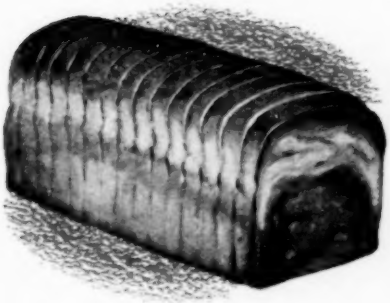

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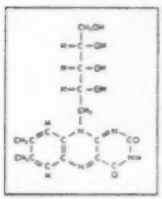
INSIDE SCIENCE

The Vital Story

A Quick History. Independent investigators, working separately to unlock several of nature's doors, sometimes open up unsuspected relationships. This happened with vitamin B_2.

Investigations. About 25 years ago, several groups, notably Warburg's, were investigating a "yellow enzyme" obtained from yeast. Almost simultaneously other investigators were studying a food factor that aided growth of laboratory animals.

What they found. Proceeding with chemical analysis of this growth factor, the team of Kuhn, György, and Wagner-Jauregg noted a relationship between the growth-producing agent and the "yellow enzyme." Their findings, and those of other researchers along similar lines, were published in 1933. Eventually, riboflavin and an essential part of the yellow enzyme were found to be identical and the unity of an essential nutrient and cellular metabolism was established.



Isolation of pure riboflavin was achieved by Kuhn and his co-workers, and by Ellinger and Koschara, in 1933.

Nomenclature. Known in the United States as riboflavin, this vitamin has also been called lactoflavin, ovoflavin, hepatoflavin, and vitamin G.

SYNTHESIS

By 1935, two eminent chemists, working separately, had synthesized riboflavin, practically in a dead heat. Prof. Paul Karrer of the University of Zurich, a collaborator of the Hoffmann-La Roche Laboratories, produced the first successful synthesis. Five weeks later Richard Kuhn of Germany announced his synthesis of the vitamin. Prof. Karrer subsequently shared the Nobel Prize in Chemistry for his work in vitamins and carotenoids.

The Karrer synthesis forms the basis for chemical processes in widespread use today by Hoffmann-La Roche and other leading manufacturers throughout the world. Riboflavin is also manufactured today by fermentation methods.

CHEMICAL AND PHYSICAL PROPERTIES

Riboflavin is yellow, slightly water-soluble with a greenish fluorescence and a bitter taste. Its empirical formula is $C_{17}H_{20}N_4O_6$. Vitamin B_2 produced by the Roche process is identical in every way with that occurring in nature.

How does vitamin B_2 work? Riboflavin is a vital part of nature's chain of reactions for utilization of carbohydrate energy. It has been found to be a constituent of many enzyme systems and is thus intimately connected with life processes. It is probably required by the metabolic processes of every animal and bird as well as by many fishes, insects and lower forms of life. (In certain animals, however, the requirement may be synthesized by bacteria within the intestine.)

In the cells riboflavin goes to work attached to a phosphate group. This substance, known as riboflavin-5'-phosphate or flavin mononucleotide, may in turn be attached to still another essential substance, adenylic acid, forming flavin adenine dinucleotide. Either nucleotide then is attached to protein, thereby forming an enzyme, and takes its part in oxidation-reduction reactions.

Requirements in Human Nutrition. As we have seen, vitamin B_2 is essential to life. We have no special storage organs in our bodies for this vitamin, although a certain level is maintained in various tissues, with relatively large amounts found in the liver and kidneys.

MEASURING METHODS

In the beginning, riboflavin activity was described in "Bourquin-Sherman units" and requirements were thought to be very small. Subsequent research showed otherwise. Milligrams of weight became the unit and the Food & Drug Administration of the U. S. Dept. of Health, Education & Welfare established (July 1, 1958) a minimum daily requirement of 1.2 mg. of riboflavin for all persons 12 or more years old. For infants it is 0.6 mg. These requirements are designed to prevent the occurrence of symptoms of riboflavin deficiency disease. The minimum daily requirement for this vitamin for children from 1 to 12 years is 0.9 milligram.

Recommended allowances. The Food & Nutrition Board of the National Research Council has recommended the following daily dietary allowances of riboflavin, expressed as milligrams. These are designed to maintain good nutrition of healthy persons in the U. S. A.

			Boys	Girls
Men			1.6	
Women			1.4	
"	(3rd trimester of pregnancy)		2.0	
"	(Lactating)		2.5	
Infants, 1-3 months			0.4	
"	4-9	"	0.7	
"	10-12	"	0.9	
Children, 1-3 years			1.0	
"	4-6	"	1.2	
"	7-9	"	1.5	
Adolescents, 10-12 years			1.8	1.8
"	13-15	"	2.1	2.0
"	16-20	"	2.5	1.9

# of VITAMIN B_2 *by* Science Writer

(Riboflavin)

Deficiencies of vitamin B_2 appear in several ways in human beings. The eyes, the skin, the nerves, and the blood show the effects of too little riboflavin. Laboratory animals have demonstrated that a riboflavin-deficient diet can cause death of adults and can slow or stop growth in the young. Female animals, deprived of riboflavin in the diet, may produce offspring with congenital malformations.

Medical uses. To overcome and control deficiencies in human beings, physicians have pure riboflavin available for administration by injection or orally, by itself or with other "B" vitamins or multi-vitamin-mineral combinations.

How do we get our daily riboflavin? Vitamin B_2 has wide distribution throughout the entire animal and vegetable kingdoms. Good sources are milk and its products, eggs, meats, legumes, green leaves and buds. Whole-grain cereals have significant but not large amounts of riboflavin.

ADDITION TO FOODS

Cereal foods play a large part in our diet. To produce the white flour almost all of us want, millers are obliged to remove parts of the wheat that contain much of the grain's riboflavin and other nutrients. In addition, cereal grains are not rich sources of riboflavin. Millers meet this problem by *enriching* the grain foods for which federal standards exist with vitamins B_1, B_2, niacin and the mineral iron. In the case of vitamin B_2, however, they do more than *restore* the processed food to its natural riboflavin level; they *fortify* the food with enough of this essential vitamin to make it nutritionally more valuable than it was in nature.

Acting to protect the good health of millions of Americans, bakers and millers adopted *enrichment* of white bread and white flour in 1941. Since that time, other foods, such as macaroni products, corn meal and grits, farina, pastina and breakfast cereals have had their food value increased by enrichment with pure riboflavin and other vitamins and minerals.

When enriching, fortifying or restoring, food manufacturers add the necessary quantity of riboflavin (and other vitamins and minerals) to the food during processing, so that the finished product meets federal, state, and territorial requirements or contributes to the consumer an amount of the vitamin that dietary experts believe significantly useful.

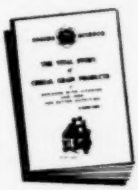
PRODUCTION

Prof. Karrer's synthesis of riboflavin was a laboratory success. Adapting the process to commercial production, however, demanded original thinking by chemists at Hoffmann-La Roche. The production of riboflavin by chemical synthesis requires the production of ribose, a rare sugar, at an early stage in the process. This special sugar must be made inexpensively if the synthesis is to be practical. Sugar chemistry is a difficult matter. In a brilliant piece of work, the Roche chemical experts developed a method to produce ribose on a commercial scale by an electrolytic process, thus overcoming a most troublesome problem. Subsequently, Roche chemists developed the first practical synthesis for riboflavin-5′-phosphate, identical with natural flavin mononucleotide.

Picture three streams joining to form a river and you have a simplified idea of the Roche process for synthesizing vitamin B_2. O-xylene and glucose are processed separately to form xylidine and ribose respectively. These are joined to form ribitylxylidine, which is then converted to ribitylaminoxylidine. Starting separately with malonic ester, which is processed through intermediate stages to alloxan, the third "stream" is then joined with ribitylaminoxylidine to form riboflavin. Purification occurs at each step of the synthesis. Riboflavin 'Roche' equals or exceeds U. S. P. standards.

By the tons. So efficient is the Roche process that pure riboflavin is produced *by the tons* for use in pharmaceutical products and processed foods. An interesting development by Roche is the production of riboflavin in different forms related to the method of end use. 'Roche' Regular riboflavin U. S. P. is especially useful in dry enrichment premixes, powdered dietary supplements, pharmaceutical tablets and soft gelatin capsules. 'Roche' Solutions type is preferred for the manufacture of solutions having low concentration. 'Roche' Riboflavin-5′-Phosphate Sodium is a highly and rapidly soluble riboflavin compound favored for all pharmaceutical liquid products and some tablets, lozenges, and capsules. It has a more pleasant taste than the bitter U. S. P. riboflavin.

This article is published in the interests of pharmaceutical manufacturers, and of food processors who make their good foods better using pure riboflavin 'Roche.' Reprints of this and others in the series will be supplied on request without charge. Also available without cost is a brochure describing the enrichment or fortification of cereal grain products with essential vitamins and minerals. These articles and the brochure have been found most helpful as sources of accurate information in brief form. Teachers especially find them useful in education. Regardless of your occupation, feel free to write for them. Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 1956 Bourdon St., St. Laurent, P. Q.



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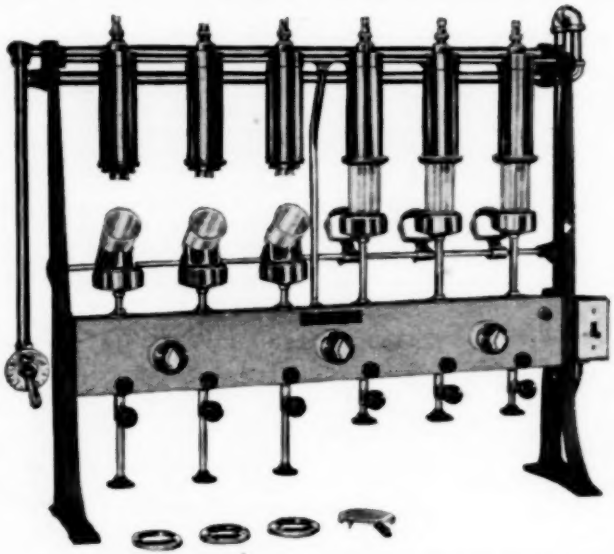

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CEREAL CHEMISTRY

VOL. 35 MAY, 1958 NO. 3

LYSINE CONTENT OF WHEAT VARIETIES, SPECIES, AND RELATED GENERA[1]

JOHN M. LAWRENCE,[2] KATHERINE M. DAY,[2]
EDITH HUEY,[3] AND BARBARA LEE[4]

ABSTRACT

Microbiological assays for lysine were carried out on 286 samples representing a broad spectrum of wheat varieties and species, related genera, and hybrids. Wheat varieties varied from a minimum of 2.46% lysine in the protein to a maximum of 3.84. Still wider variations were found among the samples of other *Triticum* species and related genera. Certain samples in each classification had proteins with a lysine content enough higher than the mean to give reason to hope that breeding wheats of consistently increased lysine content may prove to be possible.

There were no significant differences in lysine content among white spring wheats, red spring wheats, and winter wheats. The over-all mean lysine content on a unit protein basis was 2.89% for wheats of about 13.5% or more protein, but below this level there was a highly significant inverse relationship between protein content and lysine in the protein. The lysine content was not affected by the year of growth, nor by the location, except as these affect the total protein level.

Mean values for percent lysine in the protein for other groupings were, for 6 varieties of durum wheat, 2.97; for 18 samples of other *Triticum* species, 3.00; for 13 samples of genera related to wheat, 3.30; and for 15 hybrid selections of crosses of *Agropyron elongatum* with wheat, 3.10.

Feeding studies have uniformly shown that wheat protein is deficient in the amino acid, lysine (3). The practicability of breeding wheat varieties with improved lysine levels in the protein and at the same time having other desirable characteristics depends upon the existence of breeding materials with above-normal lysine. Markedly high lysine levels were not found in the seven varieties of winter wheats studied by Miller *et al.* (5). A survey of the amino acids of Russian varieties (12) showed Novo-Ukrainka 38 as best in this respect. A much more extensive survey of the lysine content of wheat breeding material

[1] Manuscript received February 5, 1957. Scientific Paper No. 1569 of the Washington Agricultural Experiment Stations, Pullman, Washington. Project No. 1273. A report of work done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract was supervised by the Western Utilization Research and Development Division of the Agricultural Research Service.

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seemed justified, especially in view of the reported (9) success in altering the lysine content of corn protein by breeding.

The following is a report of a systematic survey of the lysine contents of the proteins of a widely representative group of wheat varieties and related species and genera. In addition, a series of samples grown in different years in different locations were analyzed to determine whether the lysine level of the protein was affected by the environment.

Materials and Methods

Assays were made of 230 wheat varieties, originally developed in various parts of the world, and selected to be representative of all recognized market classes and types inventoried as cereal introduction material. In addition, 12 different *Triticum* species (other than *T. vulgare* and *T. compactum*) plus 6 varieties of durum wheats, 13 samples of genera related to wheat, mostly *Agropyron* species, and 15 hybrid selections of crosses of wheat with *Agropyron elongatum* were analyzed. Except for the study of the effect of environment, most of the samples were from wheats grown in Pullman, Washington, during the 1954 and 1955 seasons. For most of the samples of the less common *Triticum* species, and for the samples of genera related to wheat, the caryopsis had to be laboriously separated from the lemma and palea by hand. The samples were ground in a burr mill before analysis.

Hydrolysates were prepared from the ground samples by autoclaving 200 to 400 mg. with 8 ml. of 3*N* hydrochloric acid for 5 hours at 15 lb. pressure in glass-stoppered flasks (10). The hydrolysate was filtered and the residue washed. The filtrate and washings were evaporated just to dryness on the steam bath, redissolved, neutralized with potassium hydroxide, and made to a known volume for microbiological assay.

The assay organism used was *Leuconostoc mesenteroides* P-60. Difco Bacto Lysine Assay Medium (11) was used as the basal medium, to which four levels of sample in duplicate were added. With each set of assays, 32 tubes, grouped in two different locations among the tube racks and containing eight levels of lysine, were used to establish a standard curve. The cultures were carried on Difco microassay culture agar with twice-daily transfers for 2 days before being transferred to Difco microinoculum broth. The inoculum was prepared from an 18- to 20-hour culture in the latter medium. The tubes were incubated for 72 hours at 30°C. and titrated electrometrically with 0.05*N* sodium hydroxide.

In view of results such as those of Wolfe and Fowden (13), there is a need for giving evidence for the accuracy of amino acid assays.

That there were no losses of lysine during the hydrolysis and subsequent sample treatments was indicated by the following experimental results: (i) when the hydrolysis time for a wheat sample was lengthened in hourly increments from 4 to 8 hours, the mean assay results remained constant, being respectively 0.266, 0.259, 0.260, 0.247, and 0.260% lysine; (ii) removal of the hydrochloric acid used for hydrolysis by either ion exchange treatment or evaporation to dryness *in vacuo* instead of by evaporation on the steam bath resulted in no change in assay results; and (iii) triplicate assay results for wheat gluten hydrolyzed in the presence of seven parts of wheat starch were 105, 102, and 100% of that for the gluten in the absence of starch. Bovine plasma albumin plus eight parts of starch assayed 98 and 100% of the result for the albumin alone.

The reliability of the assay procedure itself was indicated by these findings: (i) recoveries of 102, 108, 103, and 98% were obtained for lysine added to wheat samples before hydrolysis; (ii) the mean assay results for wheat sample A of eight assays using *L. mesenteroides* and of two assays using *Streptococcus faecalis* were respectively 0.268 and 0.270; means for wheat sample B of four assays with *L. mesenteroides* and of two assays with *S. faecalis* were respectively 0.276 and 0.277; and (iii) assay results for different aliquot levels agreed.

All lysine assays reported represent the mean of at least two assays performed at different times on different hydrolysates. Statistical analysis of all 1,590 assays in this study showed that the standard error for a single determination in our hands was 8.2% of the actual level.

Results

The accumulated data are too extensive to be presented in their entirety in tabular form.[5] A general picture of the distribution of the assay results can be obtained from Figs. 1 to 3.

The lysine content is shown on a protein ($N \times 5.7$) basis rather than on a dry-weight or other basis, since it is the amino acid balance of the protein which is nutritionally most important. The figures for protein concentration have been corrected to a 14% moisture basis. The results for 155 varieties of spring wheats are shown in Fig. 1, and for 75 varieties of winter wheats in Fig. 2. The spring wheat varieties were found to vary from the Mexican variety, CI No. 5286, with 3.54% lysine (based on the protein content) to the variety Nanking 393, PI No. 124340, with 2.48% lysine. The over-all mean for the spring wheats was 2.91%. The maximum for the winter wheat varieties was repre-

[5] Readers interested in the results for a particular variety or varieties are invited to write to the authors for detailed analytical results.

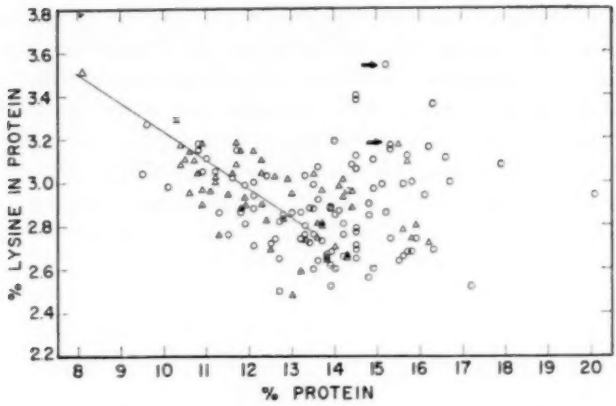

Fig. 1. Lysine content of 155 varieties of spring wheat. Each point represents the mean analysis for one sample of one variety, except where arrows point to results of analyses of two samples of the Mexican variety, CI No. 5286. Circle, red spring wheats; triangle, white spring wheats.

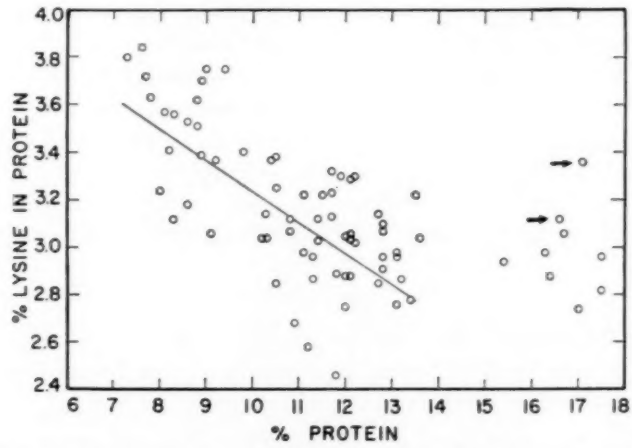

Fig. 2. Lysine content of 75 varieties of winter wheat. Each point represents the mean analysis for one sample of one variety, except where arrows point to results of analyses of two samples of the variety Wilhelmina, CI No. 4193.

sented by Golden at 3.84% lysine in the protein, and the minimum point is Tenmarq with 2.46% lysine. The over-all mean for the winter wheats was 3.15%.

Observation of the charts suggested that the lysine content of total wheat seed protein is related to the protein concentration of the seed. It appeared that the percentage of lysine increases as the protein level decreases. It seemed possible that the relatively high lysine content of

the sample of Golden with 7.6% protein might be a consequence of its low protein content. A statistical analysis to define the relationship was undertaken. Among the equations of simpler form that might be applied to the data, the lysine content of the protein considered as a function of the logarithm of the total protein content of the samples gave the best fit. The correlation was quite significant for both spring and winter wheats, but when red spring wheats were considered alone, there was no significant correlation. The explanation for this surprising result seemed to lie in the fact that most of the samples of red spring wheat varieties had protein levels in the higher range, where, perhaps, there really is no correlation. To test this, the range of protein concentrations was arbitrarily divided at 13.5% protein. Statistical analysis then showed that for all three groups—red spring, white spring, and winter wheats – there was no correlation with protein concentration for samples with more than this protein level, while there was a highly significant negative linear correlation for all three below this level. Over this latter range, the linear relationship gave just as close a correlation as the logarithmic relationship.

Furthermore, there were no significant differences among the three groups in either range. Hence they have been considered as one in calculating the statistical parameters. For samples with 13.5% protein or less the equation found for the regression curve was

$$b = 4.53 - 0.13a$$

where a = % protein (based on 14% moisture), and
b = % lysine in the protein.

The corresponding correlation coefficient was 0.73 (142 samples). The curve represented by this equation is shown in Figs. 1 and 2. For the 91 samples with more than 13.5% protein, the mean lysine content of the protein was 2.89 ± 0.21. The mean level for all samples, irrespective of protein content, was 3.10% lysine in the protein.

If one omits from consideration varieties with a low protein concentration, the spring wheat varieties with the highest lysine in the protein were the Mexican variety, CI No. 5286; Tera, PI No. 215627; a variety from India, CI No. 4127; and Siberian No. 13. It was possible to obtain a second sample of the Mexican variety, CI 5286, the most promising spring wheat variety, and of Wilhelmina, the most notable winter wheat variety. The analytical results for these varieties are indicated on the figures by arrows. In both cases, the lysine content of the protein of the second sample was lower, but was still well above the average.

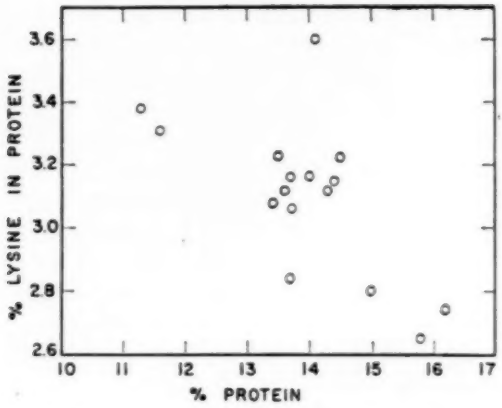

Fig. 3. Lysine content of hybrid selections from crosses of *Agropyron elongatum* with wheat.

Figure 3 shows the results of assays of 15 hybrid selections from crosses of *Agropyron elongatum* with wheat. The lysine percentage varies from a maximum of 3.60 to a minimum of 2.65, based on the protein content, with a mean of 3.10. One selection, a true-breeding octaploid derivative of the original cross, shows particular promise.

Table I gives the results for a series of *Triticum* species. The samples highest in lysine content are *T. pyramidale*, *T. sphaerococcum*, *T. persicum*, and the durum variety, Stewart. The lysine content of the genera related to wheat are listed in Table II. Those with proteins relatively high in lysine are *Secale montanum*, *Aegilops cylindrica*, *Hordeum bulbosum*, *Agropyron amurense*, *A. riparium*, and *A. trachycaulum*. There are possibilities of incorporating genetic material from most of these into ordinary wheat. The difficulty in obtaining seeds of these species, free from chaff, discouraged the effort to obtain a second sample for analysis.

Experiments were made to determine to what extent the lysine level of the protein of a particular sample is characteristic of the variety or species, or is modifiable by the environment. Three winter wheat varieties (Rio, Brevor, and Elmar) and three spring wheat varieties (Marfed, Idaed, and Baart) were grown at three different locations (Pullman and Lind, Washington, and Moro, Oregon) in three different years (1952, 1953, and 1954). In the Northwest these years showed only moderate variation from normal during the spring growing season. The year 1952 was a little warmer than normal with a rainy June; 1953 was cool and a little drier than usual. The 1954 season was a little cool, dry in early spring, and wet in late spring. The means for all

TABLE I

LYSINE CONTENT OF SPECIES OF WHEAT OTHER THAN TRITICUM VULGARE AND TRITICUM COMPACTUM

SPECIES OR VARIETY		LYSINE IN PROTEIN	PROTEIN [b]
		%	%
Triticum pyramidale	PI 117420[a]	3.50	15.1
T. pyramidale	PI 113458	3.09	15.7
T. sphaerococcum (shot wheat)	PI 190982	3.48	16.0
T. sphaerococcum (shot wheat)	PI 40944	3.04	15.9
T. persicum (Persian wheat)	PI 168672	3.31	16.3
T. persicum (Persian wheat)	PI 190948	3.24	17.5
T. turgidum (poulard wheat)		3.27	9.8
T. timopheevi	PI 119442	3.24	15.0
T. monococcum (einkorn)		3.00	16.1
T. polonicum (Polish wheat)	PI 190951	2.94	17.8
T. dicoccum (emmer)	PI 40919	2.91	16.0
T. spelta (spelt)	PI 190960	2.83	19.1
T. macha	PI 113961	2.76	18.2
T. orientale	PI 68104	2.67	14.0
T. dicoccoides (wild emmer)	B. C. Jenkins P 54.68.2	2.21	29.6
T. durum			
Stewart	CI 12066[a]	3.30	13.4
Mindum	CI 5296	3.04	12.6
Langdon	CI 13165	3.02	12.4
Ramsey	CI 13246	2.93	13.0
Sentry		2.85	13.8
Pentad (red durum)	CI 3322	2.70	12.6

[a] Plant Introduction and Cereal Investigation numbers under which plants are inventoried by the Cereal Crops Section of the Agricultural Research Service of the U. S. Department of Agriculture.
[b] 14% Moisture basis.

TABLE II

LYSINE CONTENT OF SAMPLES OF GENERA RELATED TO WHEAT

SPECIES	LYSINE IN PROTEIN	PROTEIN [a]
	%	%
Secale montanum	3.98	12.3
Hordeum bulbosum P-306 (bulbous barley grass)	3.75	17.9
Aegilops cylindrica (goat grass)	3.54	13.7
Agropyron amurense	3.50	17.4
Agropyron trachycaulum (slender wheatgrass)	3.46	20.2
Agropyron riparium — Sodar (streambank wheatgrass)	3.43	17.5
Agropyron cristatum (crested wheat grass)	3.29	16.9
Agropyron inerme (beardless wheatgrass)	3.12	21.6
Agropyron trichophorum — Lopar (pubescent wheatgrass)	3.10	18.6
Agropyron intermedium P-2327 (intermediate wheatgrass)	3.07	15.5
Agropyron elongatum P-2326 (tall wheatgrass)	3.04	19.4
Agropyron spicatum P-737 (bluebunch wheatgrass)	2.82	19.5
Agropyron repens (quackgrass)	2.80	13.4

[a] 14% Moisture basis.

varieties and stations for each of the three years, and for all varieties and years for each of three locations, were as follows:

	Lysine in Protein	*Protein*
	%	%
Location		
Pullman	3.23	11.5
Lind	3.21	12.1
Moro	3.65	7.6
Year		
1952	3.40	10.4
1953	3.36	10.6
1954	3.30	10.5

Obviously, there was no significant difference in the lysine content of the proteins of the wheats from Pullman and Lind. A covariance analysis showed that the higher lysine of the Moro wheats could be accounted for by their lower protein content. The statistical analysis also showed that there was no significant variation in the lysine of the wheat proteins grown in these three years. Miller *et al.* (5) found no difference in the lysine content of the proteins of a group of varieties grown in either the southern or the central Great Plains states.

Discussion

It is not certain how much of the variation shown in Figs. 1 and 2 is characteristic of the varieties. There was no significant variation among the six varietal means for samples grown in different locations and different years. On the other hand, the results so far suggest that Wilhelmina and the Mexican variety, CI 5286, may be truly high lysine varieties. Further analyses of additional samples of the more promising varieties are needed.

The only factor other than genetic which has been shown clearly to affect the lysine content of wheat protein is the total protein level of the sample. The inverse relationship has been pointed out by McElroy *et al.* (4), whose data were not sufficient to establish the significance of the trend, and by Price (8), who considered only four varieties. Gunthardt and McGinnis (2) found the same relation to be true when they compared the lysine content of samples of Idaed wheat whose nitrogen content was varied by fertilization. The negative correlation might be accounted for by changes in the proportion of individual proteins with different lysine contents as the total protein content changes; by changes in the lysine content of the proteins themselves; by changes in the proportion of free lysine present; or by combinations of these.

With respect to the first possibility, Barton-Wright and Moran (1) found that endosperm protein is lower in lysine than bran or germ protein. The same thing is indicated by the lower lysine content of gluten protein (7) or flour protein[6] as compared with whole-wheat

[6] Lawrence, J. M., and Day, K.; unpublished data.

protein. If low-protein wheats have a lower proportion of endosperm protein, they would be expected to have a higher percentage of lysine in their total protein. Morris, Alexander, and Pascoe (6) compared two samples and found that the one with lower protein content had a smaller proportion of endosperm proteins. To confirm this, we calculated the percentage of the total wheat protein contributed by the endosperm for a series of wheat samples of protein content ranging from 6 to 16%, using data[7] on flour yield from the Buhler mill, and protein content of the flour and whole wheat. Apparently the proportion of endosperm protein tends to be low in low-protein wheat, but not very much so—hardly enough in itself to account for the higher percentage of lysine in the total protein. In the higher-protein wheats there was no relation between the proportion of the wheat protein present in the flour and the total protein content.

We have seen no data on the relation of the lysine content of bran and germ protein to the protein content of the wheat from which it was obtained. However, it seems fairly definite that the lysine content of the endosperm protein of low-protein wheats is higher than that of higher-protein wheats. This is shown by the data of Pence *et al.* (7) on the lysine content of the crude glutens of 17 wheat varieties. The gluten lysine content was consistently higher in the low-protein flour samples. Similarly, the lysine content of flour samples from low-protein wheats is relatively high (see footnote 6). In both sets of data, the trend was not significant at higher total protein levels. Hence, it appears that not only is there relatively more high-lysine protein (from bran and germ) in low-protein wheat, but also the main protein (from endosperm) has a higher lysine content. Nothing seems to be known about the amount of lysine in the free amino nitrogen fraction of wheat, but the amount of this fraction is so small as to make it seem improbable that it can have much effect on the lysine content of wheat.

Keeping in mind the purpose of breeding wheat of higher protein quality, one should remark the fact that, in the nutritionally more valuable wheats of higher protein content, a further increase in total protein content does not imply a lower proportion of lysine. It is among these that one may seek for varieties suitable for breeding purposes.

Acknowledgments

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[7] Barmore, M. A.; personal communication.

the U. S. Department of Agriculture at Pullman, Washington. They supplied the samples for analysis, as well as valuable advice and information concerning them. We are indebted to Dr. T. S. Russell for help in the statistical analysis of the data.

Literature Cited

1. Barton-Wright, E. C., and Moran, T. The microbiological assay of amino acids. II. The distribution of amino acids in the wheat grain. Analyst **71:** 278–282 (1946).
2. Gunthardt, H., and McGinnis, J. Effect of nitrogen fertilization on amino acids in whole wheat. J. Nutrition **61:** 167–176 (1957).
3. Hegsted, D. M., Trulson, Martha F., and Stare, F. J. Role of wheat and wheat products in human nutrition. Physiol. Revs. **34:** 221–258 (1954).
4. McElroy, L. W., Clandinin, D. R., Lobay, W., and Pethybridge, S. I. Nine essential amino acids in pure varieties of wheat, barley, and oats. J. Nutrition **37:** 329–336 (1949).
5. Miller, B. S., Seiffe, Joann Y., Shellenberger, J. A., and Miller, G. D. Amino acid content of various wheat varieties. I. Cystine, lysine, methionine, and glutamic acid. Cereal Chem. **27:** 96–106 (1950).
6. Morris, V. H., Alexander, Thelma L., and Pascoe, Elizabeth D. Studies of the composition of the wheat kernel. I. Distribution of ash and protein in center sections. Cereal Chem. **22:** 351–361 (1945).
7. Pence, J. W., Mecham, D. K., Elder, Angeline H., Lewis, J. C., Snell, Neva S., and Olcott, H. S. Characterization of wheat gluten. II. Amino acid composition. Cereal Chem. **27:** 335–341 (1950).
8. Price, S. A. The amino acid composition of whole wheat in relation to its protein content. Cereal Chem. **27:** 73–74 (1950).
9. Robles, P. C. E. The microbiological assay of lysine in different varieties of corn genetically controlled. Escuela farm. (Guatemala) **13** 167/8, 15–28 (1951). (Chem. Abstracts **48:** 12933; 1954).
10. Rosenberg, H. R., and Rohdenburg, E. L. The fortification of bread with lysine. I. The loss of lysine during baking. J. Nutrition **45:** 593–598 (1951).
11. Steele, B. F., Sauberlich, H. E., Reynolds, M. S., and Baumann, C. A. Media for *Leuconostoc mesenteroides* P-60 and *Leuconostoc citrovorum* 8081. J. Biol. Chem. **177:** 533–544 (1949).
12. Taranova, A. I. Amino acid composition of wheat proteins. (In Russian.) Biokhimiya **16:** 239–245 (1951). (Chem. Abstracts **45:** 8669; 1951).
13. Wolfe, Miriam, and Fowden, L. Composition of the protein of whole maize seeds. Cereal Chem. **34:** 286–295 (1957).

CHANGES IN THE SOLUBILITY OF CORN PROTEIN RESULTING FROM THE ARTIFICIAL DRYING OF HIGH-MOISTURE CORN[1]

T. A. McGuire and F. R. Earle

ABSTRACT

The solubility of proteins in water and in 0.01*N* potassium hydroxide solution at 75° ± 5°F. (23.9° ± 2.8°C.) decreased more or less continuously with increasing drying temperatures ranging from 120° to 200°F. (48.9° to 93.3°C.); no indication was given of any critical damage occurring at any particular temperature. Some solubility measurements carried out with trichloroacetic acid and Duponol-C[2] gave a similar but less conclusive relationship with drying temperatures.

With the continued mechanical advances made in methods of harvesting corn, it has become necessary to dry artificially an increasing proportion of the U. S. corn crop. In order for the mechanical picker to operate efficiently, the corn is picked at 20–30% moisture levels. Allowing the corn to dry further in the field greatly increases picking losses (2). In addition, there is an increasing trend toward the use of the picker-sheller, which operates best when corn is at high moisture contents. Since shelled corn packs more closely and allows less circulation of air through its bulk in bins than ear corn in cribs, it dries more slowly in storage. Shelled corn at high moisture contents must be artificially dried for safe storage, except in very cold weather, or it will mold and heat.

If artificial drying of corn is carried out at too high temperatures, physical and chemical changes occur which decrease the value of the corn to dry-millers, wet-millers,[3] cattle feeders, and distillers (1, 7, 11). The decrease may result from lower yields of the desired product and/or from difficulties in plant operation and production of low-quality products. Behavior of the corn dried at high temperatures in the wet-milling process indicated that heat-treatment changed the solubility of the protein.

To gain further insight into the nature of the changes brought about in corn protein when corn was dried artificially, the solubility

[1] Manuscript received March 4, 1957. Contribution from the Northern Utilization Research and Development Division, Peoria, Illinois, one of the Divisions of the Agricultural Research Service, U. S. Department of Agriculture.

[2] Duponol-C is a product of the Dupont Company — a synthetic detergent, sodium lauryl sulfate. Mention of trade names or products does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

[3] Baird, Peggy D., MacMasters, Majel M., and Majors, K. R. Report of conference at Northern Regional Research Laboratory, January 1952.

of fractions obtained from corn dried at different temperatures was determined in several solvents.

Materials and Methods

Illinois Hybrid No. 1277 yellow dent corn, field-grown at the Illinois Agricultural Experiment Station in the years 1953, 1954, and 1955, was harvested as ear corn at approximately 20 and 30% moisture content. After shelling, the corn was dried immediately at 15 and 40% relative humidities at various temperatures between 120° and 200°F. (48.9° and 93.3°C.) to a final moisture content of about 12% (6). A control was air-dried from each initial moisture level.

The dried shelled corn received from the Illinois Agricultural Experiment Station was stored at 35°F. (1.7°C.). For the determination of the solubilities, samples were ground in a hammer mill using a screen with round holes 1/16 in. in diameter, and stored in a refrigerator. The nitrogen contents of this corn for the years 1953, 1954, and 1955, measured by the conventional Kjeldahl procedure, were 1.8, 1.9, and 1.7% respectively, on the dry basis. All experimental work reported in this paper is from the 1953 crop unless stated otherwise.

Most workers who have studied the solubilization of corn proteins have used defatted corn (5, 9, 10). For this reason, trial experiments were run on corn which had been defatted with petroleum ether at 35°F. (1.7°C.) in order to minimize the possibility of denaturing the protein during the defatting process. In studies on amino acid content of corn, Csonka (4) found that defatting the meal gave a lower value for the water-soluble nitrogenous materials than was obtained for the whole meal. Since no significant difference between the defatted meal and that of the whole corn was found here, it was decided to continue using the whole corn even though a more turbid extract was obtained.

In studying solubility differences, the procedure adopted was similar to that used by Zeleny (13). A 5-g. sample of corn was extracted with 100 ml. of the solvent by shaking for 1 hour. After shaking, the sample was centrifuged and decanted, and the supernatant was filtered through a coarse fritted-glass crucible to remove floaters which were returned to the centrifuge bottle. The residual meal was then washed three times with 50-ml. portions of solvent and the washings combined with the original extract. Later experiments showed that about the same value was obtained by making a single extraction and determining nitrogen on an aliquot, thus eliminating the necessity of running nitrogen on the whole extract and its combined washings.

To imitate the conditions used in the steeping of corn in the wet-milling industry, steepwater samples were obtained from a laboratory

processing method adapted from that used in industry (6, 8). A steepwater solution of approximately 1700 ml. was obtained, which was the total volume used in processing 400 g. of corn. Nitrogen content was determined on aliquots from the steepwater from the processing of the 1955 series.

Experimental and Results

In studying differences in the solubility of nitrogen of artificially dried and air-dried corn, preliminary comparisons were made between the samples dried at 200°F. (93.3°C.) and the air-dried control. If no significant difference between these two samples was found with a particular solvent, no further work was done with that solvent on the series of samples.

Extraction with 60% ethyl alcohol at 175°F. (79.4°C.) without previous extraction of the corn showed no difference in nitrogen extracted from the air-dried and from the samples dried at 200°F. (93.3°C.).

Results of successive extractions made with water, 5% sodium chloride, 60% ethyl alcohol at 175°F. (79.4°C.), and 0.2% potassium hydroxide on a sample dried at 200°F. (93.3°C.) and an air-dried control are shown in Table I. The table indicates that the greatest differences were found in the water-soluble and 5% sodium chloride-soluble fractions. No consistent advantages were observed in using either 5% sodium chloride or water as extractant. Water was used as the extractant on the whole series because it was more convenient.

TABLE I

PERCENT OF TOTAL NITROGEN EXTRACTED WITH SUCCESSIVE SOLVENTS FROM ARTIFICIALLY DRIED AND AIR-DRIED CORN

SOLVENT	DRIED AT 200°F. (93.3°C.)	AIR-DRIED
1. Water[a]	12.2	15.8
2. 5% Sodium chloride[a]	3.7	7.2
3. 60% Ethanol[b]	40.7	38.3
4. Aqueous potassium hydroxide[a]	17.2	19.2
Residual meal	25.8	18.9
Total	99.6	99.4

[a] Extractions made at room temperature — 75°F. ± 5°F. (23.9°C. ± 2.8°C.).
[b] Extractions made at 175°F. (79.4°C.).

Figure 1 shows the decrease in the water-soluble nitrogen content with increase in temperature of drying air. As shown here, the water-soluble nitrogen content decreased with increased drying temperature, regardless of whether the corn was harvested at 30 or 20% moisture levels or whether it was dried at 15 or 40% relative humidity. The points in Fig. 1 represent averages of two or more determinations. The

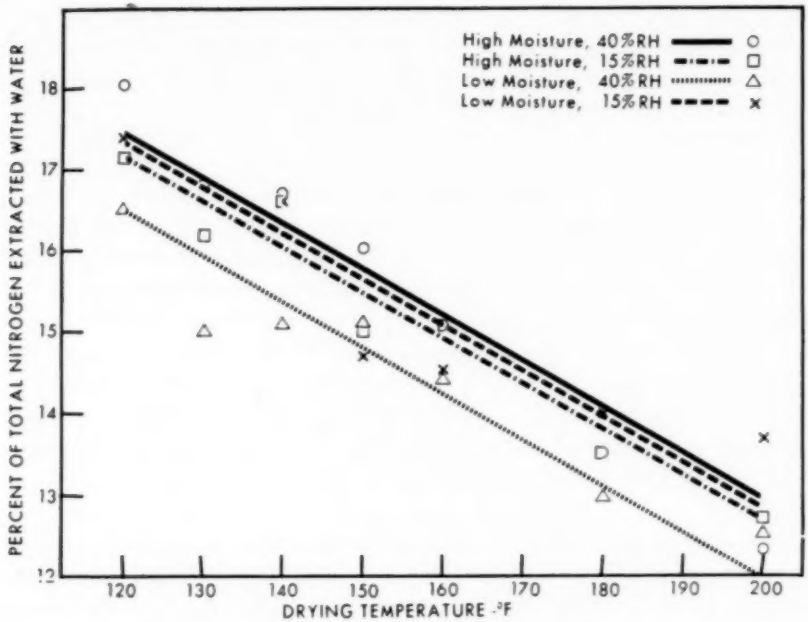

Fig. 1. Change in percent of total nitrogen extractable by water from corn with change in temperature of drying air.

spread between duplicates of 14 samples extracted with water averaged 0.44%, with an extreme of 1.0%. Analysis of the data in Fig. 1 showed the individual regression coefficients were not statistically different and the four parallel regression lines are the best representation of the data. The three top regression lines differ only slightly; however, the bottom line indicates that the solubility of protein for low moisture corn dried at 40% relative humidity is smaller, indicating possibly a greater denaturation.

Table II shows the variations which may be encountered in total nitrogen extractable with water from the air-dried sample and the samples artificially dried at the highest and lowest temperatures in different crop years. The 1953 crop shows the highest percentage of water-extractable nitrogen and the 1954 crop the lowest. Analysis of variance showed the mean difference between years was highly significant. The difference between the mean of the air-dried controls and the mean of the artificially dried samples was highly significant, as was the difference between the 120°F. (48.9°C.) mean and the 200°F. (93.3°C.) mean. Differences between initial moisture and relative humidity means were nonsignificant. All interactions were nonsignificant.

TABLE II

VARIATION IN PERCENT OF TOTAL NITROGEN EXTRACTED WITH WATER FROM CORN DRYING SERIES IN DIFFERENT YEARS

Initial Moisture	Relative Humidity	Drying Temperature	Total Nitrogen Extracted		
			1953	1954	1955
%	%	°F. (°C.)	%	%	%
30	...	Air-dried	15.8	15.7	15.8
20	...	Air-dried	18.3	13.4	16.8
30	40	120 (48.9)	18.1	14.3	15.9
30	15	120 (48.9)	17.4	13.8	16.5
20	40	120 (48.9)	16.5	13.7	16.5
20	15	120 (48.9)	17.7	13.9	15.4
30	40	200 (93.3)	12.3	9.2	9.6
30	15	200 (93.3)	12.7	9.6	11.4
20	40	200 (93.3)	12.6	8.4	10.7
20	15	200 (93.3)	13.7	9.1	12.3

Although the complete series was not run, the 1954 and 1955 crop results follow the same trend as shown in Fig. 1 for the 1953 crop.

After preliminary studies were made with alkali of various strengths on the air-dried and the sample dried at 200°F. (93.3°C.), it was decided to use 0.01*N* potassium hydroxide to study the differences among the whole series of samples. The results of these extractions are shown in Fig. 2. The points shown in the figure represent averages of two or

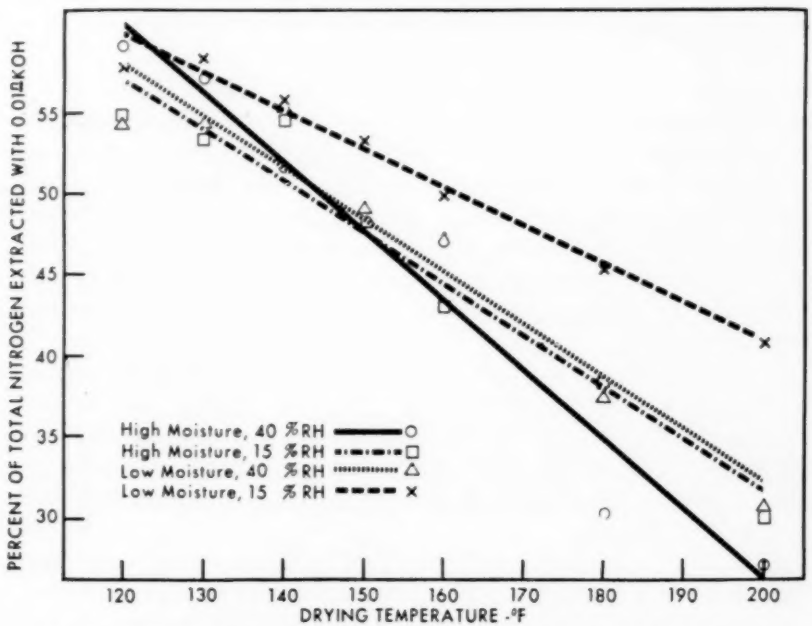

Fig. 2. Change in percent of total nitrogen extractable with 0.01*N* potassium hydroxide from corn with change in temperature of drying air.

more determinations. The spread between duplicates of 21 samples averaged 0.89% with an extreme of 2.6%. Analysis of the data indicated a significant difference among regression coefficients. These data are best represented by the regression lines plotted in Fig. 2. Because of the large differences shown between the higher and lower drying temperatures in Fig. 2, it may be assumed that other proteins, in addition to those solubilized with water, are also denatured at the higher drying temperatures.

Table III shows the variation from year to year of the percentage of the total nitrogen extractable with 0.01*N* potassium hydroxide from

TABLE III

VARIATION IN PERCENT OF TOTAL NITROGEN EXTRACTED WITH 0.01*N* POTASSIUM HYDROXIDE FROM CORN DRYING SERIES IN DIFFERENT YEARS

Initial Moisture	Relative Humidity	Drying Temperature	Total Nitrogen Extracted		
			1953	1954	1955
%	%	°F. (°C.)	%	%	%
30	..	Air-dried	56.6	45.0	51.6
20	..	Air-dried	51.5	45.8	45.0
30	40	120 (48.9)	58.8	46.7	55.4
30	15	120 (48.9)	54.8	45.6	51.8
20	40	120 (48.9)	54.1	45.7	46.6
20	15	120 (48.9)	57.6	45.4	46.3
30	40	200 (93.3)	27.2	29.6	27.6
30	15	200 (93.3)	30.5	30.5	33.8
20	40	200 (93.3)	30.9	26.1	33.5
20	15	200 (93.3)	41.1	28.6	35.6

the air-dried samples and the samples artificially dried at the highest and lowest temperatures. Upon analysis of these data the same conclusions were drawn as for the water extraction shown in Table II, except for a slight moisture by temperature interaction. This interaction was barely significant at the 0.05 probability level.

Attempts to measure differences by extraction with a weak sulfur dioxide solution, such as had been done with other solvents, were not very satisfactory.

Figure 3 shows the results of the nitrogen extractable with the laboratory wet-milling process. The data from Fig. 3 are extremely variable. Analysis indicated that the regression coefficients were not significantly different. Likewise, differences among group means were not significant. Therefore, a single regression line was plotted to represent these data.

The regression analyses showed that initial moisture and relative humidity did have some effect on extractable nitrogen. However, these analyses are based on data from one year only. The analyses based on data from Tables II and III indicated that initial moisture and rela-

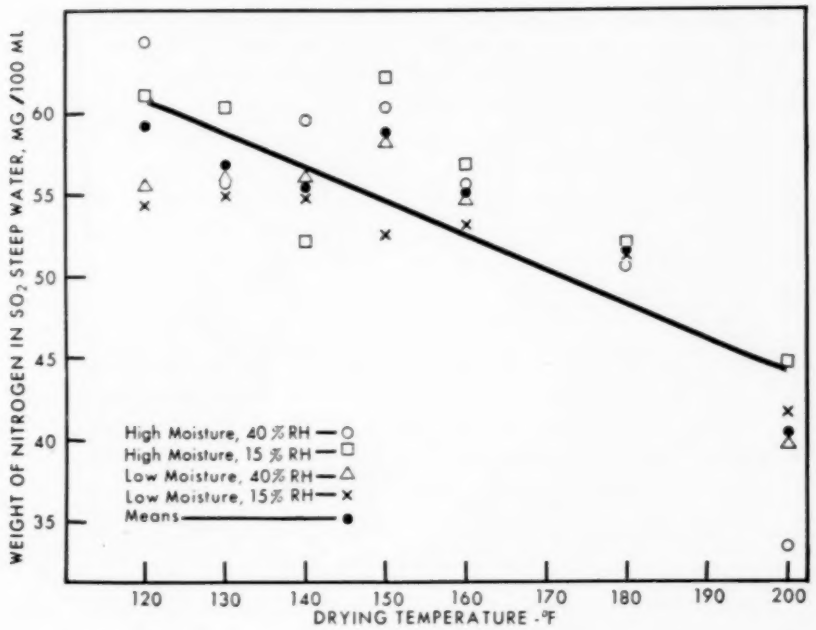

Fig. 3. Change in aqueous sulfur dioxide-extractable nitrogen from corn with change in temperature of drying air.

tive humidity were essentially ineffective. This apparent discrepancy was probably due to the fact that the year-to-year variability was greater than that caused by moisture and relative humidity.

Trichloroacetic acid had been used by Smith and Circle (12) and Becker *et al.* (3) to show the effect of concentration upon the solubilization of soybean proteins. Their work showed that the protein-precipitating action of the acid was very dependent upon the concentration. A sample of corn, Schwenk 13, was used for studying the effects of concentration variations. This sample contained 1.46% nitrogen (dry basis), of which 13.5% was extractable with water. Figure 4 shows the variations in the amount of nitrogen extracted from this corn, plotted on a semilogarithmic scale against the concentration of trichloroacetic acid. This curve is similar to that obtained by Becker *et al.* (3) on soybean meal. Only with very high concentrations of trichloroacetic acid was more nitrogen extracted than with water. Because of this fact there was little possibility to show any large differences between the air-dried and artificially dried corn except at the higher concentrations. However, in order to determine the concentration of trichloroacetic acid which would show the greatest difference

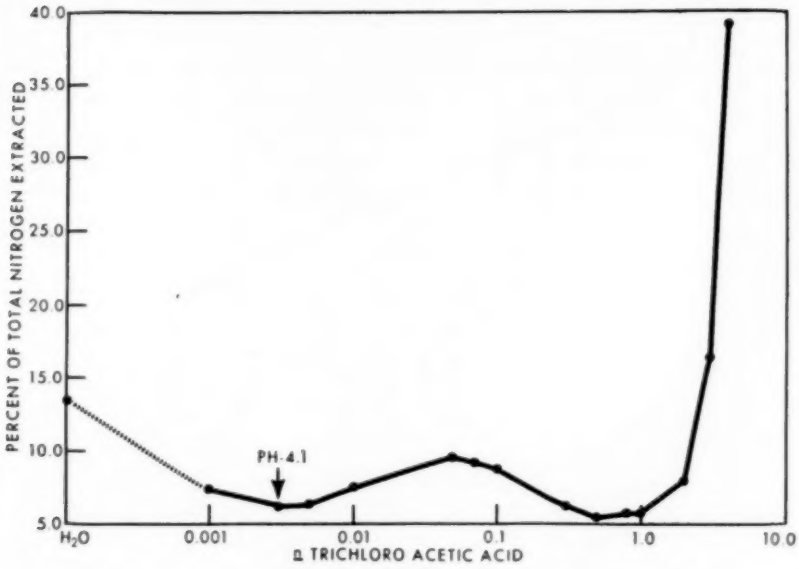

Fig. 4. Change in percent of total nitrogen extractable from a hybrid corn — Schwenk 13 — with change in concentration of trichloroacetic acid.

between the amount of nitrogen extracted from corn dried at 200°F. (93.3°C.) and an air-dried sample, several concentrations were tried. The results of these extractions are given in Table IV. With the two

TABLE IV

PERCENT OF TOTAL NITROGEN EXTRACTED FROM CORN AIR-DRIED AND ARTIFICIALLY DRIED AT 200°F. (93.3°C.) WITH VARIOUS CONCENTRATIONS OF TRICHLOROACETIC ACID

N TRICHLOROACETIC ACID	TOTAL NITROGEN EXTRACTED	
	Artificially Dried 200°F. (93.3°C.)	Air-Dried
	%	%
0.05	7.4	9.2
0.1	7.3	8.9
0.8	5.3	5.6
4.0	38.0	43.8

lower concentrations, small differences were found, while at the 0.8*N* concentration the values are lower and practically the same, which was to be expected. With a 4.0*N* concentration the values obtained had a difference of 5.8 percentage points, which would not be of as much value as using water or 0.01*N* potassium hydroxide.

Detergents have also been used to extract corn proteins (5). In the

present study, experiments were made using 5 g. of corn in 100 ml. of 1% Duponol-C in 0.01*N* potassium hydroxide; it was noticed that the sample dried at 200°F. (93.3°C.) could be dispersed rather uniformly by stirring, but it settled quite rapidly, whereas the air-dried sample gummed up somewhat in a sticky mass. Continued stirring tended to decrease the difference between the two samples. The Duponol-C solution removed 80% of the nitrogen from the artificially dried sample and about 88% from the air-dried sample.

An attempt was made to show the relationship between the physical consistency of the corn in the detergent with the drying temperature. Samples of the corn with high initial moisture content, dried at 40% relative humidity, were used. In general, it could be concluded that the samples dried at the lower temperatures were more gummy in appearance when stirred with the detergent than those dried at the higher temperatures. It was not possible, however, to detect any difference between samples dried only 10°F. apart. Protein denatured by heat may be responsible for a progressive decrease in the tendency to gum. In attempting to determine these physical differences, the rate of settling was measured on this series of samples. With a concentration of 2 g. per 25 ml., the samples were thoroughly stirred and allowed to stand for 30 minutes. The difference was not great enough between successive samples to be significant.

Conclusions

Measurements of the solubility of the protein fraction of corn in water, 0.01*N* potassium hydroxide, and a sulfur dioxide steep showed qualitatively that denaturation, or at least changes in the physical state of the protein, takes place with increasing drying temperatures. No discontinuity in solubility was found which might show a critical temperature limit for safe drying operation.

Acknowledgments

The corn samples used in these experiments were obtained under an informal memorandum of understanding with the Illinois Agricultural Experiment Station. The Agricultural Engineering Department of the Illinois Station grows, harvests, and dries the corn under known, controlled conditions. The samples are processed at the Northern Division under the direction of Dr. Majel M. MacMasters to determine their suitability for wet-milling for starch production. The authors wish to thank Dr. Morris D. Finkner, Biometrician, Biometrical Services, Plant Industry Station, Beltsville, Maryland, for the excellent statistical treatment given the data in this paper. The authors also wish to thank Mrs. Margaret Holzapfel for supplying the sulfur dioxide steepwater samples.

Literature Cited

1. Adams, S. L., Stark, W. H., and Kolochov, P. Reduction of the fermentable carbohydrate content of corn by kiln drying. Cereal Chem. **20:** 260–266 (1943).

2. BATEMAN, H. P., PICKARD, G. E., and BOWERS, W. Corn picker operation to save corn and hands. Univ. Ill. Agr. Ext. Service Circ. 697 (August 1952).
3. BECKER, H. C., MILNER, R. T., and NAGEL, R. H. A method for the determination of nonprotein nitrogen in soybean meal. Cereal Chem. **17:** 447–457 (1940).
4. CSONKA, F. A. Amino acids in the corn kernel. J. Agr. Research **59:** 765–768 (1939).
5. FOSTER, J. F., YANG, J. T., and YUI, N. H. Extraction and electrophoretic analysis of the proteins of corn. Cereal Chem. **27:** 477–487 (1950).
6. GAUSMAN, H. W., RAMSER, J. H., DUNGAN, G. H., EARLE, F. R., MACMASTERS, MAJEL M., HALL, H. H., and BAIRD, PEGGY D. Some effects of artificial drying of corn grain. Plant Physiol. **27:** 794–802 (1952).
7. HATHAWAY, I. L., YUNG, F. D., and KIESSELBACH, T. A. The effect of drying temperature upon the nutritive value and commercial grade of corn. J. Animal Sci. **11:** 430–440 (1952).
8. MACMASTERS, MAJEL M., EARLE, F. R., HALL, H. H., RAMSER, J. H., and DUNGAN, G. H. Studies on the effect of drying conditions upon the composition and suitability for wet milling of artificially dried corn. Cereal Chem. **31:** 451–461 (1954).
9. NAGY, D., WEIDLEIN, W., and HIXON, R. Factors affecting the solubility of corn proteins. Cereal Chem. **18:** 514–523 (1941).
10. SHOWALTER, M. F., and CARR, R. H. Characteristic proteins in high and low protein corn. J. Am. Chem. Soc. **44:** 2019–2023 (1922).
11. SIMMS, B. T., Chief, Bureau of Animal Industry in Agricultural Research Service Reports p. 115 (1949), p. 151 (1950), p. 156 (1951).
12. SMITH, A. K., and CIRCLE, S. J. Peptization of soybean proteins. Ind. Eng. Chem. **30:** 1414–1418 (1938).
13. ZELENY, L. The distribution of nitrogen in the seed of *Zea mays* at different stages of maturity. Cereal Chem. **12:** 536–542 (1935).

A MODIFICATION OF THE EXTENSOGRAPH FOR STUDY OF THE RELAXATION OF EXTERNALLY APPLIED STRESS IN WHEAT DOUGH[1]

BEN GROGG[2] AND DIANE MELMS[2]

ABSTRACT

A contact switch has been installed on an Extensograph® which stops the downward movement of the dough hook at a variable but predetermined dough extension. The kymograph continues running after the dough hook stops, resulting in the formation of a relaxation curve. This modification permits the use of the instrument as a "standard" relaxometer to provide direct comparison of results by various investigators. Collaborative studies of stress decay may also be made.

A method of treating the experimental data based on the Central Limit Theorem is presented which permits the investigator to study the distribution characteristics of the so-called "spectrum" of Maxwell elements in wheat doughs.

Several investigators (1, 3, 4, 5) have studied the relaxation of external stress in wheat flour doughs by special instruments which they have developed. However, few, if any, laboratories make routine investigations of factors which influence the relaxation of externally applied stresses in doughs. Such investigations would be of great practical value since the major uses of wheat flour are dependent upon the viscoelastic behavior of dough.

The Extensograph® is a relatively common instrument used by cereal chemists, and a number of papers have described its function of measuring the extensibility of dough. A simple attachment has been devised, and is described herein, which permits the dough cylinder to be extended a predetermined amount and then records the decrease in stress as a function of time.

Whitby (6) applied the Central Limit Theorem to an analysis of the stress relaxation data of Cunningham *et al.* (1). The relaxation data obtained with the Extensograph are used to present additional details on the application of the Central Limit Theorem.

A brief study of the effect of varying the absorption and sodium chloride content of the dough has been made to demonstrate 1) the modification of the instrument and 2) the statistical method of analyzing the accumulated data.

Materials and Methods

The same doughs were used in this study as were reported in an

[1] Manuscript received January 15, 1957.
[2] The Quaker Oats Company Research Laboratories, Barrington, Ill.
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earlier paper (2). These doughs were prepared from an unbleached hard winter wheat straight-grade flour using 58, 60, 62, and 64% absorption and 0, 1, 2, 3, and 4% sodium chloride (based on weight of flour). Mixing was done in a National experimental dough mixer for 200 g. flour.

The doughs were scaled to 150 g., rounded 20 turns, molded, saddled, and placed in the Extensograph cabinet for a 30-minute rest period prior to stretching. All materials and instruments were maintained at 25°C.

Modification of the Extensograph

The modification is shown in Fig. 1 and consists of a contact switch which stops the downward movement of the dough hook at a predetermined, but variable, position while allowing the continued movement of the chart paper so that a relaxation curve is drawn. A metal strip, attached to the dough hook, closes the electrical circuit upon con-

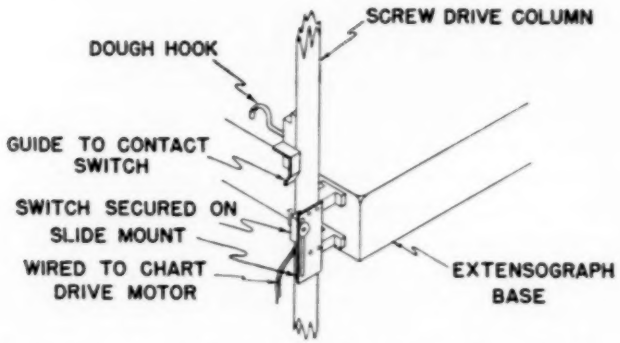

Fig. 1. Isometric drawing of the contact switch positioned on the Extensograph.

tact with the switch. The switch is of the type used for starting the movement of the chart paper. It is wired through the circuit normally used for stopping the upward movement of the dough hook at its uppermost position. For conventional Extensograph operation the circuit is shorted, allowing the dough hook to travel downward until the dough cylinder is broken. The only sacrifice of such an arrangement is the automatic stopping of the dough hook upon its return to the top of the screw drive. The contact switch is mounted on a slide and secured by a set-screw, allowing the positioning of the switch to give variable extensions of the dough cylinder.

The damping device was loosened so it would not interfere with the response of the pen arm.

This modification does not compensate for the dough carrier displacement as the tension or resistance to extension is increased. The contact switch can be positioned to stop the dough hook at a fixed dough extension, as indicated by the chart paper, but this does not give all doughs a constant extension, since the distance the dough is actually stretched becomes a function of the tension. The tension is measured by mechanical magnification of the dough carrrier displacement. Thus a dough which offers a large resistance to extension (large tension) is actually stretched a lesser amount than a dough which offers less resistance to extension. After relaxation all doughs are stretched equally. While this error is appreciable it is systematic and has not been considered in this study.

An extension of 5 cm., as indicated by the chart paper, was used and the doughs were allowed to relax for 12.5 cm. (18.75 seconds).

Results and Discussion

In Fig. 2 typical curves obtained with the modified Extensograph are shown. These curves may be transformed by plotting tension vs. the

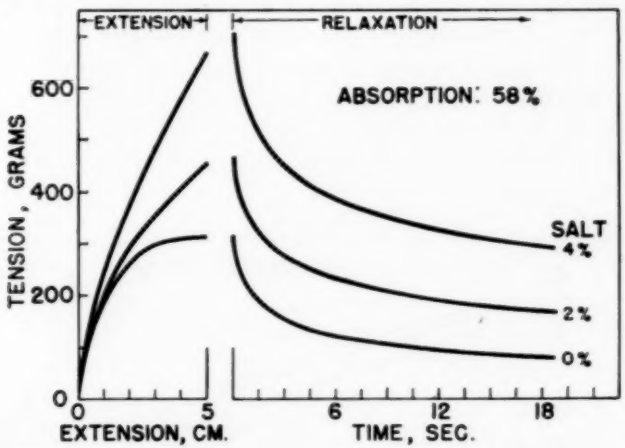

Fig. 2. Typical extension and relaxation curves formed with modified Extensograph. In practice the curves are continuous but are broken here to show a change from length to time dimensions.

logarithm of time over the relaxation range as shown in Fig. 3, and analyzed by the graphical estimation of the distribution function (1). Such an analysis leads to the conclusion that wheat flour doughs contain a continuous array of Maxwell elements, and thus a continuous distribution of relaxation times. Under such conditions one may apply the Central Limit Theorem as suggested by Whitby (6), thus avoiding

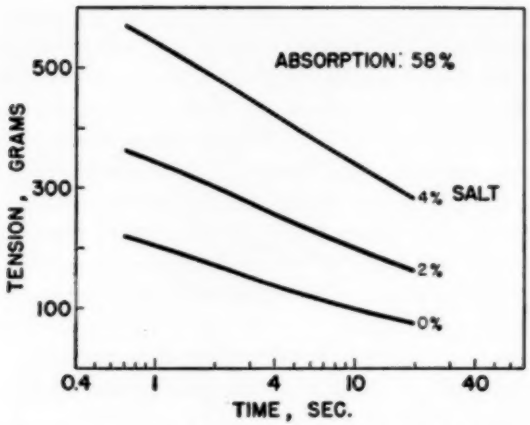

Fig. 3. Classical treatment of relaxation curves. Stress is plotted against the logarithm of time.

the tediousness and inherent error of calculating the instantaneous slope and replotting, as suggested by Cunningham *et al.* (1). To apply the Central Limit Theorem, the stress at various time intervals is calculated as a percent of the initial stress (peak of the continuous curve formed) and replotted on probability coordinates against the logarithm of time. In Fig. 4 the same curves shown in Figs. 2 and 3 are plotted in this manner. From such a plot the geometric mean and geometric

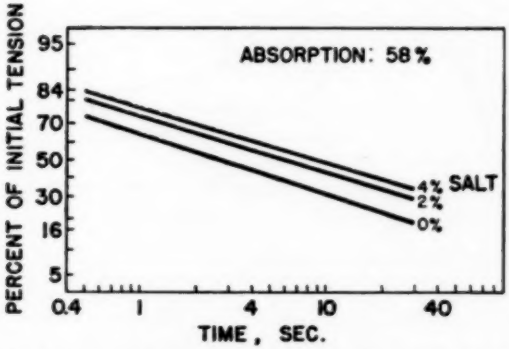

Fig. 4. Statistical treatment of relaxation curves. Percent of initial stress plotted on probability coordinate against the logarithm of time.

standard deviation of relaxation times of the distribution of Maxwell elements may be estimated graphically. The geometric mean is the time value at the 50% stress decay level; the geometric standard de-

viation is the ratio of the time values at the 50 and 84% and/or the 16 and 50% stress decay levels. The geometric mean and standard deviation of relaxation times for the various samples tested are given in Table I. The methods used for estimation are further explained in the appendix.

TABLE I

EFFECT OF ABSORPTION AND SODIUM CHLORIDE LEVEL ON RELAXATION CONSTANTS OF DOUGH

Absorption	Sodium Chloride, Percent of Flour				
	0.0	1.0	2.0	3.0	4.0
%	Geometric mean, sec.				
58	2.7	4.0	5.8	6.8	8.8
60	2.6	4.0	6.0	6.5	7.4
62	2.4	3.8	4.7	5.8	6.5
64	2.4	3.1	4.0	5.0	6.2
	Geometric Standard Deviation				
58	14.8	16.5	18.4	18.2	19.4
60	13.8	20.3	18.3	18.5	19.5
62	14.2	17.4	18.1	18.6	18.5
64	13.6	14.8	16.3	17.2	18.1
	Coefficient of Variability				
58	2.7	2.0	1.6	1.5	1.4
60	2.7	2.2	1.6	1.6	1.5
62	3.0	2.1	1.9	1.7	1.6
64	3.0	2.4	2.0	1.8	1.6

When no sodium chloride is present in the dough, a slight decrease in the mean relaxation time was noted as the absorption was increased from 58 to 64%. With 4% sodium chloride in the dough, the mean relaxation time was appreciably longer and decreased at a greater rate over the absorption range than was noted for unsalted doughs. Intermediate sodium chloride levels gave intermediate results.

The geometric standard deviation of relaxation times followed the same general pattern as the geometric mean when the absorption and sodium chloride level were varied. The coefficient of variability was calculated for each mean and standard deviation (in logarithms) and the values are given in Table I. The method of calculation is further explained in the appendix. When the absorption was increased at a given salt level, the coefficient of variability remained essentially constant, indicating that the mean and standard deviation vary in direct proportion. When the salt level was increased, the coefficient of vari-

ability decreased, indicating that the mean increased at a greater rate than the standard deviation.

This simple modification of the Extensograph permits the formation of dough relaxation curves which can be subjected to collaborative study. The statistical treatment of the relaxation data provides a precise mathematical means of characterizing the so-called spectrum of Maxwell elements in wheat doughs.

Appendix

In Table II the chart-paper travel, equivalent time interval, tension, and percent tension are given for the curve formed when 58% absorption and 4% sodium chloride level were used. The chart paper had previously been calibrated in grams by noting the pen arm deflection brought about by dead loading of the dough carrier. The time at which maximum height of the extension curve was obtained was taken as zero time of the relaxation curve, and the maximum height obtained was used as the tension at zero time.

Application of the Central Limit Theorem assumes a linear relationship between the logarithm of relaxation time and the probit of percent tension. The regression of probit percent tension upon logarithm of time may be calculated by standard statistical methods. The geometric mean relaxation time or the time required for decay of 50% of the tension and the geometric standard deviation may be found by appropriate substitution into the regression equation.

$$y = mx + b$$

where y = probit percent tension

x = logarithm time (t) in seconds

Then solution of the equation at $y = 4.0$, 5.0, and 6.0 will give the logarithm of relaxation time at 16, 50, and 84% tension respectively. The geometric mean relaxation time is then:

Antilog t at $y = 5.0$

and the geometric standard deviation will be:

Antilog t of: log t at $y = 6.0$ minus log t at $y = 5.0$;

or: log t at $y = 5.0$ minus log t at $y = 4.0$

A simplified graphical method was used for estimation of the geometric mean and standard deviation. The percent tension was plotted against the relaxation time on logarithmic normal paper (Codex No. 31, 376 or 32, 376). A straight line was fitted by eye and the geometric mean relaxation time determined by reading the time at the 50% tension level. The geometric standard deviation may be approximated from the ratio:

time at 16% tension/time at 50% tension
or time at 50% tension/time at 84% tension.

The coefficient of variability is then found by:

C.V. = log geometric standard deviation/log geometric mean.

These constants have been calculated at the bottom of the table.

TABLE II

EXAMPLE CALCULATION
(58% absorption; 4% sodium chloride)

CHART PAPER TRAVEL	RELAXATION TIME	TENSION	TENSION
cm	*seconds*	g	%
0	0	704	100
0.5	0.75	565	80.3
1.0	1.50	508	72.2
1.5	2.25	474	67.3
2.0	3.00	446	63.4
2.5	3.75	426	60.5
3.5	5.25	395	56.1
5.0	7.50	366	52.0
7.0	10.50	335	47.6
8.5	14.25	310	44.0
12.5	18.75	288	40.9

By graphical estimation:

Relaxation time at 84% tension: 0.45 seconds
Relaxation time at 50% tension: 8.8 seconds
Relaxation time at 16% tension: 171.0 seconds
Geometric mean relaxation time: 8.8 seconds
Geometric standard deviation:
8.8/0.45: 19.4 seconds
171/8.8 : 19.4 seconds

Coefficient of Variability = log 19.4/log 8.8 = 1.36

Literature Cited

1. CUNNINGHAM, J. R., HLYNKA, I., and ANDERSON, J. A. An improved relaxometer for viscoelastic substances applied to the study of wheat dough. Can. J. Technol. **32:** 98–108 (1953).
2. GROGG, B., and MELMS, D. A method of analyzing extensograms of dough. Cereal Chem. **33:** 310–314 (1956).
3. HALTON, P. Significance of load-extension tests in assessing the baking quality of wheat flour doughs. Cereal Chem. **26:** 24–45 (1949).
4. HALTON, P., and SCOTT BLAIR, G. W. A study of some physical properties of flour doughs in relation to their breadmaking qualities. Cereal Chem. **14:** 201–219 (1937).
5. HLYNKA, I., and ANDERSON, J. A. Relaxation of tension in stretched dough. Can. J. Technol. **30:** 198–210 (1952).
6. WHITBY, K. T. A statistical approach to the mechanisms of certain physical phenomena. U. of Minn. Institute of Technology, Mechanical Engineering Department, Minneapolis, Minnesota.

GELATINIZATION OF STARCHY MATERIALS IN THE FARINOGRAPH[1]

BEN GROGG AND E. F. CALDWELL

ABSTRACT

Use of a farinograph equipped for operation at 100°C. is proposed as a means of evaluating the thermal gelatinization characteristics of starchy materials at higher solids-water ratios than are possible in the amylograph. A wheat starch dough consisting of equal parts of starch and water reached maximum consistency in 2 minutes when mixed in the farinograph at 100°C. Additions of methyl oleate caused the attainment of this maximum to be delayed by an interval which was approximately proportional to the amount of ester added. A similar but more striking effect resulted from the addition of oat oil to starch. The gelatinization time of oat flour doughs mixed at 100°C. appeared to be proportional to the fat content of the oat flour used.

The thermal gelatinization or swelling characteristics of starchy materials have been the subject of much investigation. Harris and Sibbitt (5) have shown that the swelling power of starch is increased by methanol extraction. Relatively low concentrations of starch were present in the suspensions used in their experiments. The same is also true of the suspensions used for amylograph measurements. Little work has been done with concentrations high enough to result in cooked doughs suitable for extrusion or other unit operations.

In the present study a farinograph was equipped for operation at 100°C. and was employed to investigate the influence of different levels of methyl oleate and of oat oil on the gelatinization characteristics of a concentrated wheat starch suspension. The effect of oat oil content on the farinograms of oat flour suspensions was also investigated. Some comparative studies were made with the amylograph.

Methods and Materials

Construction and use of the farinograph have been described by previous authors (2,4,6,9), but in nearly all cases its use has been confined to measurements on wheat flour doughs at 30°C. In the present studies, a Brabender high-temperature pressure thermostat was used which permitted the doughing of material at temperatures of up to 160°C., employing ethylene glycol as the circulating liquid. The mixing bowl used was the micro or "50-g." size. Bowl and blades were constructed of stainless steel.

The standard procedure for doughing starchy materials was as fol-

[1] Manuscript received May 10, 1957. Contribution from The Quaker Oats Company, John Stuart Research Laboratories, Barrington, Ill.

lows: Mixing bowl temperature was brought to 100°C. Sensitivity adjustment was set in the second or middle position, and the system adjusted to read zero with the mixer running empty. Thirty-three grams (dry basis) of the starchy material were placed in the bowl, and water added to a predetermined absorption level, counting the sample moisture as a part of the dough water. The glass cover plate was put in place, using a few drops of water as a seal, and the instrument allowed to run long enough to establish a characteristic pattern.

Farinograms were first prepared by the standard method from wheat starch alone and with various increments of purified methyl oleate. A water absorption of 100.0% was used, calculated as a percentage of the dry weight of the starch. The starch used was General Mills' "Aytex C" ground through a 1/16-in. r.h.p. screen in a laboratory hammermill. The methyl oleate was prepared by esterifying oleic acid and methanol, the product being purified by double distillation under high vacuum and stored in evacuated ampoules under refrigeration.

Farinograms were also prepared from wheat starch with small increments of oat oil, using the same water absorption as before. The oil was prepared by extracting oat groats overnight with petroleum ether in a large Soxhlet apparatus and subsequently recovering the oil by distilling off the solvent.

To contrast the farinograph technique with the amylograph procedure which has been used to study starch gelatinization (1), amylograms were prepared from mixtures of wheat starch and methyl oleate. In each case, 45 g. (dry basis) of starch were slurried with 450 ml. of water and heated from 30°C. to 96°C. at the rate of 1.5°C. per minute. To ensure conditions reasonably comparable with those of the farinograph experiments, the methyl oleate was incorporated in the starch by gentle mulling in a glass mortar prior to preparation of the water suspension.

Several farinograms were also prepared from oat flours of varying fat contents. Oat flour having a natural crude fat content of 6.5% was extracted overnight with petroleum ether in a large Soxhlet apparatus to give a flour with 0.8% crude fat. These extracted and unextracted flours then formed the extremes of a fat content series, the intermediate members of which were made up by mixing extracted and unextracted flours to give the desired intermediate fat contents and thus simulate partial extraction. A water absorption of 80.0% was used in obtaining farinograms of these flours.

Results and Discussion

Farinograms of the starch-methyl oleate series are shown in Fig. 1,

curves A–D. "Development" of a plastic dough with starch alone occurred within a period of 2.0–2.5 minutes, as indicated by the peak in curve A. Additions of the purified ester delayed dough development in proportion to the amount added, as shown by curves B, C, and D.

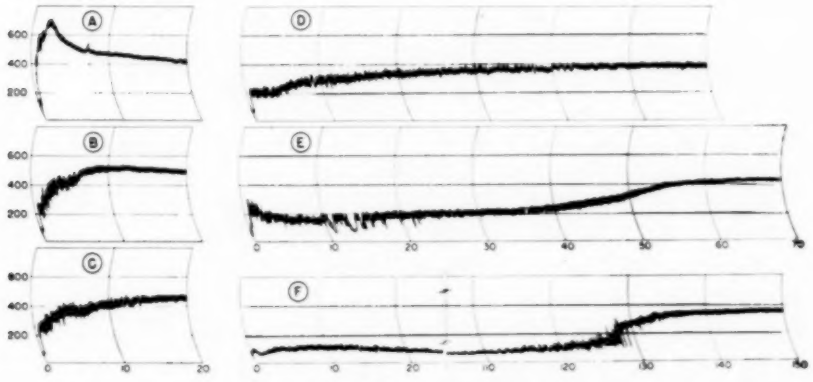

Fig. 1. Farinograms of wheat starch at 100°C. and 100.0% water absorption, showing effect of added fat and fatty acid ester. A, control; B, 3% methyl oleate; C, 6% methyl oleate; D, 12% methyl oleate; E, 1.5% oat oil; F, 3.0% oat oil.

Farinograms of the starch-oat oil series are also shown in Fig. 1, curves E and F. Addition of even a small amount of oil delayed development markedly, and larger amounts appeared to postpone it almost indefinitely.

Amylograms of starch and starch plus methyl oleate are shown superimposed on one another in Fig. 2. They show little effect resulting from the addition of fatty acid ester to wheat starch.

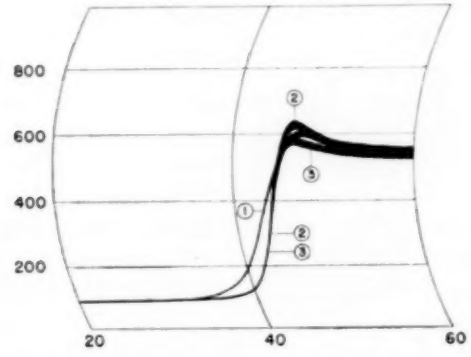

Fig. 2. Amylograms of 10% suspensions of wheat starch, showing the effect of added fatty acid ester. Curve 1, control; curve 2, 6% methyl oleate; curve 3, 12% methyl oleate. Percentage of methyl oleate stated by weight of starch.

Farinograms of the oat flour series appear in Fig. 3. With extracted oat flour at 0.8% fat, an immediate development occurred as shown in curve A. This contrasts sharply with the delayed development of normal oat flour at 6.5% fat as shown in curve D. Intermediate development times were shown by the mixed flours with intermediate fat contents.

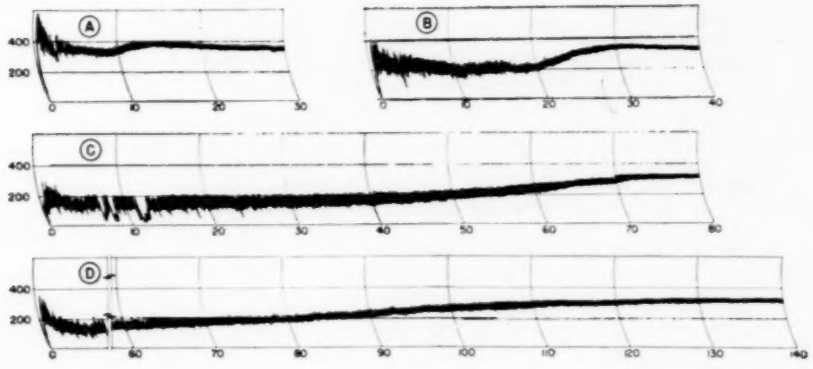

Fig. 3. Farinograms of oat flour at 100°C., and 80.0% water absorption, showing the influence of fat content. A, 0.8% fat; B, 2.7% fat; C, 4.6% fat; D, 6.5% fat. Flour for curve A was solvent-extracted. Flour for curve D was untreated, its natural content of oat oil being 6.5%. Flours for curves B and C were made by mixing extracted and unextracted material in proportions of 2:1 and 1:2 respectively.

The delaying of dough development caused by the presence of fatty ester or triglyceride, represented in these studies by methyl oleate and oat oil, may be caused by the starch being kept from contact with water or by a simple "lubricating" effect. In either case, eventual development of a plastic dough, presumably as a result of starch gelatinization, might be explained on the basis of absorption of fatty acid chains into starch helices, as proposed by Mikus, Hixon, and Rundle (8).

Use of the farinograph for mixing doughs at cooking temperatures has been described primarily for its value as a technique for investigating the gelatinization or swelling characteristics of starchy materials. The data presented to illustrate the method indicate that differences in the "cooking" characteristics of starchy materials can be determined by means of the farinograph just as can differences in the mixing characteristics of wheat flour. Development time has been found to be unaffected by changes in the water absorption level within the range in which a plastic dough is produced.

In addition to the farinogram itself, another useful aspect of the

high-temperature method is that the resulting dough may be used to make small quantities of cooked cereal products by extrusion (3) or flaking (7). A small-scale method is thus provided for testing the effect of changes in formula or processing technique on flavor, texture, or shelf life of cooked cereals.

Literature Cited

1. Anker, C. A., and Geddes, W. F. Gelatinization studies upon wheat and other starches with the amylograph. Cereal Chem. **21:** 335–360 (1944).
2. Brabender, C. W. Studies with the farinograph for predicting the most suitable types of American export wheat and flours for mixing with European soft wheats and flours. Cereal Chem. **9:** 617–627 (1932).
3. Campbell, F. D. Extrusion of cooked plastic material. U. S. Patent No. 2,036,681 (1936).
4. Geddes, W. F., Aitken, T. R., and Fisher, M. H. The relation between the normal farinogram and the baking strength of Western Canadian wheat. Cereal Chem. **17:** 528–551 (1940).
5. Harris, R. H., and Sibbitt, L. D. Some effects on swelling power of wheat starch of (1) Soxhlet extraction with methanol and (2) composition of the cooking liquid. Food Research **14:** 190 (1949).
6. Johnson, J. A., Shellenberger, J. A., and Swanson, C. O. Farinograms and mixograms as a means of evaluating flours for specific uses. Cereal Chem. **23:** 388–399 (1946).
7. Lilly, E. F., and Rupp, E. G. Process for making oat cereal flakes. U. S. Patent No. 2,552,290 (1951).
8. Mikus, F. F., Hixon, R. M., and Rundle, R. E. The complexes of fatty acids with amylose. J. Am. Chem. Soc. **68:** 1115–1123 (1946).
9. Swanson, C. O. Physical properties of dough. Burgess Pub. Co.: Minneapolis, Minn. (1943).

RELATIONSHIPS AMONG DOUGH INGREDIENTS, WATER ABSORPTION, MOISTURE CONTENT OF BREAD, AND BREAD YIELD[1]

JOAN BARBER and BARBARA M. KENNEDY

ABSTRACT

Calcium caseinate, heat-treated soluble milk albumin solids, and calcium peroxide had water absorptions of over 2 g. per g. of solids. With other ingredients, water absorption varied from 0.08 to 1.74 g.

Losses due to proofing, baking, and cooling, and amount of water in bread were increased slightly in two of the flours with the addition of 6, 12, and 18% heat-treated, nonfat dry milk. Proofing, baking, and cooling losses were significantly lower than those in the control loaves when nonfat dry milk, soluble milk albumin solids, and whey, all with no heat treatment, were added. These losses were higher than the control when calcium caseinate was added.

Percent water in bread was increased with all of the dough ingredients tried, except whey. Percent of added water retained in the bread was in the same range as that in the control doughs with varying water absorption, for all of the dough ingredients except soluble milk albumin solids, partially delactosed, nonfat dry milk, soy flour, and calcium peroxide. With these ingredients, the percent of added water retained in the bread increased.

The greatest increases in bread yield per g. of ingredient were obtained with calcium peroxide, heated soluble milk albumin solids, and calcium caseinate.

Water constitutes about 40 to 50% of the bread dough mass. Dough colloids with high water-binding capacities decrease the rate of water evaporation and the loss of weight during baking. Skovholt and Bailey (23) used the term "bound water" to indicate the difference between total and free water. Free water exists in a dough as water that can dissolve substances added to it with normal change in colligative properties and that is therefore easily lost by heat, as contrasted with bound water that is held in colloidal combination with starch and protein and is less easily removed in baking. The bound-water content of flour-water doughs has been variously estimated as 28.2% (3), 35.5% (28), and 51.4% (23) of the total dough water. The relation of milk solids to water-binding capacity and baking loss is not entirely clear, and relatively little work is reported in the literature. Skovholt and Bailey (23) reported a hydration capacity of 62.1% for moisture-free, nonfat dry milk as compared with 43.1% for the flour solids in the same system.

A reasonable amount of bread solids is one of the standards established by the Federal Food and Drug Administration for bread quality.

[1] Manuscript received June 3, 1957. Contribution from the Department of Nutrition and Home Economics, University of California, Berkeley. The data in this paper are taken in part from a thesis presented to the Graduate Division of the University of California by Joan Barber in partial fulfillment of the requirements for the degree of Master of Science.

To ensure this, the Administration, in its "Definitions and Standards of Identity" (27), has set a maximum limitation of 38%, by weight, upon the water content of bread. The purpose of this study is to note the relationships of nonfat dry milk (and other high moisture-absorbing ingredients of doughs) and variable baking conditions to baking loss, bread yield, and moisture content of bread, and to evaluate the latter factor in relation to federal bread standards.

Materials and Methods

A series of breads was made with and without various experimental ingredients. The effects of those ingredients on water absorption, dough and bread yield, proofing, baking, and cooling losses, moisture content of bread, and loaf volume were studied. As a basis for comparing the effects of added water when experimental ingredients were added, the water absorption was varied in the doughs made from flour B with no added ingredient. Breads were made using 62.0, 63.5, 65.0, and 66.0% water absorption, and the loaves were baked at 210° and 244°C. Loaves with 63.5% water absorption were also baked at 221° and 232°C.

In addition to material obtained from the experimental breads, scaling practices, baking losses, oven temperature, and other data were obtained from two commercial bakeries. Representative samples of different types of bread were weighed, at the bakeries, when 1 hour out of the oven, and moisture determinations were made on some of these loaves after they were brought back to the laboratory.

Ingredients. Three bread flours, described in Table I, were used.

TABLE I
DESCRIPTION OF FLOURS

Flour	Grade	Type and State Where Grown	Bromated	Bleaching Treatment	Protein [a] (N × 5.7)	Ash [a]	Water Absorption [a]
					%	%	%
A	83% patent	60% Baart; Washington 40% hard red winters; Montana and Washington	No	Chlorine dioxide and benzoyl peroxide	10.8	0.40	58.0
B	80% patent	Spring and winter; Montana and Washington	No	Bleached	11.6	0.39	63.5
WW	Whole wheat	Spring Montana	No	Chlorine dioxide	14.6	1.46	66.5

[a] 14% Moisture basis.

A spray-dried, heat-treated commercial nonfat dry milk of good baking quality was added at levels of 0, 6, 12, and 18%. With flours B and A, baking temperatures of 210°, 221°, 232°, and 244°C. were used, and with flour WW 210°C. only. An experimentally spray-dried sample of nonfat dry milk, not preheated, containing 37.5% protein and 6.3% soluble whey protein, was used at levels of 6 and 12% with flour B at baking temperatures of 210°, 221°, and 232°C.

Various milk fractions were studied. Commercial calcium caseinate containing 93.2% protein and 4.8% ash, commercial bulk whey, not preheated, and partially delactosed, nonfat dry milk (48.5% protein, 11.7% ash, 33.4% lactose), a commercial product with no preheat treatment, were added at levels of 6 and 12%. Commercial soluble milk albumin solids, not preheated (83.1% protein, 10.7% ash) was added at 3 and 6% levels, and heat-treated, soluble milk albumin (prepared by adding water to the unheated product and heating at 88°C. for 20 minutes) was added in a semisolid form at 3, 6, and 12% levels. Doughs were made with flour B and baked at 210°C.

Flour and starch products, 6 and 12%, used were: full-fat, "cooked" soy flour obtained from a commercial bakery; sweet rice flour; potato flour; corn starch; and wheat starch. Calcium peroxide was also included at the 0.5% level. Doughs were made with flour B and baked at 210°C.

Experimental Baking Test. The baking procedure followed that of Kennedy *et al.* (13), using the dough formula with 2% yeast. Optimum fermentation and proofing times were determined by varying the basic schedule and observing volume and texture of the baked loaves. The times used were as follows:

	Flour WW	*Flour B*	*Flour A*
	minutes	*minutes*	*minutes*
First punch	115	155	105
Second punch and scaling	45	40	50
Molding and panning	20	20	15
Total fermentation time	180	215	170
Proofing time	65	90	65

The absorption values were determined from the handling characteristics of doughs to which increasing amounts of water were added. Up to a point, it was possible to add more water by extending the mixing time, but properties of the fermented dough were the final criteria. Optimum water absorption was taken to be that amount giving a soft, pliable dough out of the mixer, which, when fermented, was not too sticky to handle or to put through the sheeting rolls. A few additional

milliliters of water often made little difference in properties of dough as it came from the mixer, but significantly increased the stickiness of the fermented dough. This was particularly true of doughs containing 12% nonfat dry milk which had received no preheat treatment. Halton (11) found that fermentation time and water content are, to a large extent, interchangeable in their effects, so that the terms "optimum" or "correct" absorption and "optimum fermentation" are only relative, not absolute.

Optimum mixing times were used. Doughs were mixed until they were fully developed, as shown by smooth texture and a dry, elastic feel when handled. This stage was considered to be reached at 1 minute past the time required to clear the sides and bottom of the mixer. In a trial series in which doughs were mixed for longer and shorter times, no effect on loaf volume was observed so long as the doughs were adequately developed but not mixed long enough to show definite signs of breakdown in the mixer.

Doughs were scaled to 150 g. or 495 g. The smaller loaves were baked for 20 minutes at 232°C., and the 495-g. loaves for 30 minutes at 210°, 221°, 232°, or 244°C. (410°, 430°, 450°, or 470°F.). Most of the baking was conducted at 210°C., since baking losses at that temperature were comparable with commercial baking losses. The single, most important factor in obtaining good checks, among duplicates, on baking loss and moisture content of the bread was the maintaining of uniform oven heating conditions. Standardized thermometers placed in the oven were read during the baking period. The loaves were spaced at regular time intervals, and placed one to a shelf. A pan of hot water was kept in the oven during baking. Loaves were cooled for 1 hour after removal from the oven. They were then weighed, and the volume was measured in a volumeter.

Percentages of experimental ingredients added were calculated on a flour weight basis, and corrections were made for moisture variation. Weights of milk solids or milk products were adjusted to a 3.5% moisture basis, and all flours and starches to a 14% moisture basis, so that dry weights of milk samples and flours, respectively, were the same. Levels of test materials used were 3, 6, 12, and 18%.

Duplicate or triplicate bakes were made on loaves from which basic deductions were to be made. Interday variation among duplicates was no greater than that among loaves baked the same day. Single loaves only were baked in some cases where no differences in oven loss were shown, as compared with control loaves, or where values followed a consistent trend.

Moisture Determinations. Moisture in the dough ingredients was

determined by the aluminum-plate method (1). Nonfat milk solids were heated for 4 minutes, a time period which gave values that checked with the vacuum-oven method. Moisture in the dough was calculated from the moisture content and the weight of the ingredients.

Moisture in bread was determined by the procedure described in *Cereal Laboratory Methods* (1) except that the aluminum-plate oven at 140° C., instead of a vacuum oven or air oven, was used for drying the air-dried crumbs. With 2-g. samples, a 25-minute period in the aluminum-plate oven gave results comparable with those obtained in the air oven. Bread containing little or no milk solids gave good checks by the two methods, but bread containing high levels of milk solids darkened considerably and gave higher results by the air oven. Although the aluminum-plate oven temperature was 140°C., the more rapid heat transfer permitted faster drying (and evidently less decomposition of the milk solids) during 25 minutes than was achieved during 1 hour at 130°C. in the air oven. Duplicate determinations were consistently much closer by the aluminum-plate than by the air-oven method – another reason for using the former. Sieber (21) and Walker (29) also noted that the official methods were not accurate for drying bread containing milk solids.

Calculations. Increase in dough and in bread yield was calculated from the weights of the control dough and the dough containing the added ingredient, and the weights of the control bread and the bread containing the added ingredient. Proofing, baking, and cooling losses were determined from the weight of the loaf immediately after panning and the weight of the baked loaf after 1 hour of cooling at room temperature. In a few cases the loaves were weighed just before they were placed in the oven instead of after panning, and baking and cooling losses were calculated. The percentage of dough moisture that remained in the baked bread was calculated from the total amount of water in the dough and the total amount of water in the bread.To test the moisture-retaining capacity of the ingredient, the percentage of water added to the dough with the ingredient and retained in the bread was calculated. This was done by comparing the difference in the dough water in the control dough and in the dough with the added ingredient with the difference in water in the control bread and in the bread with the added ingredient.

Effect of Type of Pan and Dough Weight on Baking Loss. In preliminary work, it was noted that the weight of the dough had a marked effect on baking loss. Therefore, an experiment was carried out to see how the weight of the dough and the type of pan affected the baking and cooling losses and the moisture in bread. Loaves of 150 g. and of

495 g., made from the basic formula (flour B) with no added ingredient and with 6% nonfat dry milk, were baked at 232°C., 20 minutes for the 150-g. doughs and 30 minutes for the 495-g. doughs. The 150-g. doughs were baked in low-form 2xx tin pans, 9.5 by 5.5 cm. bottom dimensions, and 5.0 cm. deep, and the 495-g. doughs in tin pans 9.0 by 19.0 cm. bottom dimensions, 7.0 cm. deep, and weighing 233 g. The 495-g. doughs containing the milk solids were also baked in tin pans of the same dimensions, but of three different weights: 210 g. (pan corrugated), 233 g., and 415 g. Results are shown in Table II.

TABLE II

EFFECT OF PAN WEIGHT AND DOUGH WEIGHT ON BAKING AND COOLING LOSS AND MOISTURE CONTENT OF BREAD (FLOUR B)[a]

Pans: Inside Dimensions Bottom	Pans: Inside Dimensions Depth	Weight	Scaled Dough Weight	Number of Loaves	Water: In Dough	Water: In Bread	Baking and Cooling Loss
cm	*cm*	*g*	*g*		%	%	%
Basic formula							
5.5 × 9.0[b]	5.5	90	150	2	43.8	34.5	15.2
9.0 × 19.0	7.0	233	495	2	43.8	35.7	13.9
Commercial nonfat dry milk, 6%							
5.5 × 9.0[b]	5.5	90	150	4	44.3	34.8	15.7
9.0 × 19.0[c]	7.0	210	495	2	44.3	36.6	13.5
9.0 × 19.0	7.0	233	495	1	44.3	36.1	14.2
9.0 × 19.0	7.0	415	495	2	44.3	35.8	14.6

[a] Baking temperature, 232°C.; baking time, 20 minutes for 150-g. doughs, 30 minutes for 495-g. doughs.
[b] Low-form, 2xx tin.
[c] Corrugated.

The baking treatment of the 150-g. loaves was more severe, in relation to their size, than was that of the large loaves: 150-g. doughs lost a higher percentage of their total weight, and their moisture content was lower than that of the larger loaves although the baking time was 10 minutes shorter. The rate of temperature increase in bread doughs is a function of the size of the dough mass as well as of oven temperature (4). The moisture variation among duplicate loaves from 150 g. of dough was as great as 0.9%, which was much higher than in the larger loaves. Because of this variability, and because 495-g. doughs more closely approximate commercial practice, the remainder of the experiments was carried out with the larger dough weight.

Bakery losses were least in the lightest-weight tin. There was an increase of from 13.5% to 14.6%, with an increase of 215 g. in pan weight, and the moisture content of the bread dropped from 36.6% to 35.8%. The loaves described in the remainder of this paper were baked in the heavy-weight pans.

Results and Discussion

Effect of Varying the Water Absorption. Although smaller than that used by other workers (6, 15, 24), the 4% absorption range used in this experiment is probably typical of the range in dough consistency which might be encountered in bakery practice. The highest absorption dough (66.0%) was quite slack; addition of more water would have made it too sticky to handle.

All of the doughs baked at 210° and 244°C. gave increased dough and bread yields and increased percentages of water in the bread when the water absorption was increased (Table III). These increases have been noted by previous workers: dough yield (9, 24); bread yield (9, 15, 24); moisture in bread (6, 9, 24).

TABLE III

EFFECT OF WATER ABSORPTION AND OF BAKING TEMPERATURE ON THE MOISTURE CONTENT OF BREAD AND ON BREAD YIELD (FLOUR B)

Baking Temperature [a]	Water Absorption	Water		Weight of Dough After Mixing [b]	Weight of Bread	Increase in Yield		Proofing, Baking and Cooling Loss	Added Water in Dough Found in Bread
		In Dough	In Bread			Dough	Bread		
°C	%	%	%	g	g	%	%	%	%
210	62.0	44.6	37.7	512	440	0.0	0.0	13.2	73 [c]
210	63.5	45.9	38.0	516	443	0.8	0.6	13.3	69
210	65.0	45.4	38.6	520	446	1.6	1.4	13.4	80
210	66.0	45.8	39.0	524	450	2.3	2.3	13.3	80
210	63.5	45.0	3.80	516	443	0.0	0.0	13.3	...
221	63.5	45.0	37.2	516	436	0.0	—1.4	14.5	...
232	63.5	45.0	36.7	516	433	0.0	—2.2	15.2	...
244	63.5	45.0	35.8	516	427	0.0	—3.5	16.4	...
244	62.0	44.6	35.5	512	425	0.0	0.0	16.2	66 [c]
244	63.5	45.0	35.8	516	427	0.8	0.5	16.4	51
244	65.0	45.4	36.1	520	430	1.6	1.2	16.6	52
244	66.0	45.8	36.5	524	432	2.3	1.7	16.8	57

[a] Baking time, 30 minutes. Doughs scaled to 495 g.
[b] Calculated from weight of ingredients.
[c] Percent of total water in dough found in bread.

Doughs baked at 210°C. showed no significant increase in proofing, baking, and cooling losses with increasing absorptions, the difference between the lowest and the highest losses being only 0.1%. With a more severe bake (244°C.), increases in absorption resulted in slight increases in baking loss, the largest difference being 0.6%. Of the proofing, baking, and cooling losses, proofing losses accounted for from 0.4 to 0.8%. Proofing losses were not correlated with changes in water absorption. Varying the water absorption had no effect on cooling losses in breads baked at 210° and 244°C. Cooling losses ranged from 1.1 to 1.8% in 14 loaves. The losses were larger (1.6 to 1.8%) in bread baked

at 210°C., and smaller (1.1 to 1.3%) in bread baked at 244°C. Other workers have not agreed on the effect of absorption variation on baking loss. Cathcart (6) found that baking losses were affected little by different absorptions, while Micka and Child (15), Skovholt and Garnatz (24), and Garnatz and Kornreich (9) reported a slight increase in oven loss with increase in absorption. The latter felt that losses need not increase if absolute control of oven conditions were attainable.

The percent of added water in the dough that was later found in the bread did not vary greatly with increase in absorption (69 to 80% at 210°C.; 51 to 57% at 244°C.), but was less in the more severe bake. Skovholt (and Garnatz, 24) reported 21, 11, and 68% of added water lost when water absorption was increased. Skovholt concluded that extra baking, achieved through increased baking time, will tend to drive off a larger proportion of the water in wetter doughs. The data in the present paper indicate that more severe baking, effected through higher baking temperatures rather than increased baking time, did not drive off a larger proportion of the water in wetter doughs since, at 244°C., 51% of the added water was found in the bread when the absorption was 63.5% and 57% when the absorption was 66%.

Among the loaves with 62.0, 63.5, and 65.0% water absorption, average loaf volumes varied only 10 cc. at 210°C. and 20 cc. at 244°C. With 66.0% water absorption, loaf volumes decreased 100 cc. from the control (63.5% water absorption) at 210°C., and 55 cc. from the control at 244°C.

At a given water absorption (63.5%), higher baking temperatures, as might be expected, produced increased baking losses and lower bread yield and moisture content (Table III).

Nonfat Dry Milk. The effects of a commercial, heat-treated, nonfat dry milk and of a nonheat-treated, nonfat dry milk on water absorption, dough and bread yield, proofing, baking, and cooling losses, moisture content of bread, and loaf volume are shown in Table IV.

(a) Water absorption: With flours WW and B, one part water was added with each part dry milk at all levels, and very satisfactory doughs were obtained. However, with flour A, which had the lowest water absorption of the three flours, this amount of water could not be used without producing sticky doughs. The parts of water per part of dry milk were therefore decreased as the amount of milk was increased (Table IV). One part water per part nonfat dry milk of good baking quality has been recommended by several workers (7, 19, 25). Shipstead (20) reported ratios of water to milk of from 0.58 to 1.50 for 6% milk doughs, depending on the amount of heat treatment given the milk.

(b) Dough and bread yield: When sufficient water was incorporated

TABLE IV

EFFECT OF NONFAT DRY MILK AND OF BAKING TEMPERATURE ON PROOFING, BAKING, AND COOLING LOSS AND ON BREAD YIELD

Flour and Baking Temperature		Water Absorption	Nonfat Dry Milk, Heat-Treated		Weight of Dough [a]	Weight of Bread	Increase in Yield Over No-Milk Control		Proofing, Baking, and Cooling Loss
			Absorption				Dough	Bread	
	°C	%	%	g. water / g. milk	g	g	%	%	%
WW	210	66.5	0	...	525	460	...	...	11.5
		72.5	6	1.05	561	490	6.6	6.4	11.8
		79.0	12	1.05	597	522	13.5	13.4	11.7
		83.0	18	0.96	629	549	19.6	19.4	11.8
B	210	63.5	0	...	516	443	...	...	13.3
		69.5	6	1.05	552	472	7.0	6.6	13.6
		75.5	12	1.05	587	504	13.8	13.8	13.4
		80.0	18	0.96	618	532	19.8	20.3	13.0
		65.5	6 [b]	0.38	540	464	4.6	5.6	13.2
		64.0	12 [b]	0.08	553	483	7.2	9.0	11.9
B	221	63.5	0	...	516	436	...	...	14.5
		69.5	6	1.05	552	466	7.0	6.7	14.7
		75.5	12	1.05	587	495	13.8	13.4	14.8
		65.5	6 [b]	0.38	540	460	4.6	5.5	13.8
		64.0	12 [b]	0.08	553	480	7.2	10.0	12.4
B	232	63.5	0	...	516	433	...	...	15.2
		69.5	6	1.05	552	459	7.0	6.0	16.0
		75.5	12	1.05	587	490	13.8	13.2	15.7
		65.5	6 [b]	0.38	540	453	4.6	4.6	15.3
		64.0	12 [b]	0.08	553	474	7.2	9.5	13.5
B	244	63.5	0	...	516	427	...	...	16.4
		69.5	6	1.05	552	453	7.0	6.0	17.1
		75.5	12	1.05	587	483	13.8	13.0	17.0
		80.0	18	0.96	618	510	19.8	19.3	16.8
A	210	58.0	0	...	521	447	...	...	13.4
		63.5	6	0.90	557	474	6.8	6.2	13.9
		65.0	12	0.60	581	499	11.4	11.8	13.1
		65.0	18	0.40	599	519	15.0	16.1	12.6
A	221	58.0	0	...	521	441	...	...	14.6
		63.5	6	0.90	557	471	6.8	6.8	14.6
		65.0	12	0.60	581	493	11.4	11.9	14.2
		65.0	18	0.40	599	515	15.0	16.8	13.3
A	232	58.0	0	...	521	435	...	...	15.8
		63.5	6	0.90	557	466	6.8	7.2	15.4
		65.0	12	0.60	581	487	11.4	12.1	15.2
		65.0	18	0.40	599	509	15.0	17.0	14.3
A	244	58.0	0	...	521	427	...	...	17.2
		63.5	6	0.90	557	458	6.8	7.3	16.7
		65.0	12	0.60	581	480	11.4	12.2	16.6
		65.0	18	0.40	599	500	15.0	17.0	15.7

[a] Calculated from weight of ingredients.
[b] Not heat-treated.

into the doughs, the percent increase in bread yield of the milk loaves was very close to that of the dough (Table IV). When insufficient water

was used, as was necessary with flour A with 12 and 18% milk solids, and with the nonheat-treated dry milk, bread yield was greater than the dough yield because of the lower proofing, baking, and cooling losses. In these cases, however, both dough and bread yields were lower than with flours WW and B with the heat-treated milk solids. Increases in dough and bread yield were largest for flours WW and B with 18% heat-treated milk.

(c) Proofing, baking, and cooling losses, given in Table IV, were lower for whole-wheat flour WW than for the white flours B and A. With flours WW and B, the addition of the heat-treated dry milk tended to increase these losses slightly at any given baking temperature. Addition of the nonheat-treated milk solids tended to decrease proofing, baking, and cooling losses when 6% solids were added, and to lower the loss considerably when 12% solids were added. In the latter case, however, the water absorption of the milk was very low. With flour A, the water absorption of the heat-treated milk was lower than with flour B, and decreased as the percent of milk increased. Proofing, baking, and cooling losses were decreased with the addition of milk, at all baking temperatures, except for 6% milk at 210° and 221°C. Skovholt and Garnatz (24) reported no difference in baking loss, and Platt (17) reported a decrease when nonfat dry milk was increased in the dough.

(d) Moisture content of bread: The question has been raised as to whether or not the inclusion of milk solids in the baking formula will decrease the percentage of moisture lost during the baking process. Can the addition of milk solids bring the moisture content of the bread over the 38% limit set by the Federal Food and Drug Administration? Percent water in the bread (Table V) was increased, with flours WW and B, for any given baking temperature, by the addition of heat-treated milk solids. With flour A, percent water in the bread was increased with 6% milk, but decreased with 12 and 18% milk where it was necessary to lower the water absorption of the milk. Percent water in bread also decreased with nonheat-treated milk solids (flour B) where the water absorption of the milk was lower than that of the flour. Whole-wheat bread, with or without nonfat dry milk, baked at 210°C. was well over 38% moisture and, while white water bread made from flour B and baked at 210°C. was close to 38% moisture, the addition of dry milk increased the moisture content as much as 0.8% with 12 and 18% milk. Platt (17) reported a very slight increase in moisture for milk bread, 36.8% for water bread, and 37% for a super-milk bread with 35% powdered whole milk.

(e) Water lost during baking: The percent of dough water found in

TABLE V

EFFECT OF NONFAT DRY MILK AND OF BAKING TEMPERATURE ON THE MOISTURE CONTENT OF BREAD

Flour and Baking Temperature		Water Absorption	Nonfat Dry Milk, Heat-Treated	Water in Dough[a]		Water in Bread		Added Water in Dough Found in Bread
	°C	%	%	%	g	%	g	%
WW	210	66.5	0	45.0	242	40.4	186	77[c]
		72.5	6	46.3	260	40.6	199	71
		79.0	12	46.6	278	40.8	213	74
		83.0	18	46.4	292	40.4	222	72
B	210	63.5	0	45.0	232	38.0	168	73[c]
		69.5	6	45.4	250	38.4	181	69
		75.5	12	45.7	269	38.8	196	74
		80.0	18	45.7	282	38.8	207	76
		65.5	6[b]	44.2	239	37.6	174	87
		64.0	12[b]	42.4	235	36.0	174	183
B	221	63.5	0	45.0	232	37.2	162	70[c]
		69.5	6	45.4	250	37.6	175	69
		75.5	12	45.7	269	37.8	187	67
		65.5	6[b]	44.2	239	36.7	169	97
		64.0	12[b]	42.4	235	35.3	170	248
B	232	63.5	0	45.0	232	36.7	159	68[c]
		69.5	6	45.4	250	36.8	169	54
		75.5	12	45.7	269	37.2	182	64
		65.5	6[b]	44.2	239	35.9	163	59
		64.0	12[b]	42.4	235	35.1	166	262
B	244	63.5	0	45.0	232	35.8	153	66[c]
		69.5	6	45.4	250	35.9	162	53
		75.5	12	45.7	269	36.3	175	61
		80.0	18	45.7	282	36.2	184	62
A	210	58.0	0	43.8	228	36.5	163	72[c]
		63.5	6	44.1	245	36.9	175	69
		65.0	12	43.3	252	36.5	182	81
		65.0	18	42.1	252	35.3	183	82
A	221	58.0	0	43.8	228	35.9	158	69[c]
		63.5	6	44.1	245	35.9	169	62
		65.0	12	43.3	252	35.2	174	65
		65.0	18	42.1	252	34.6	178	83
A	232	58.0	0	43.8	228	34.8	151	66[c]
		63.5	6	44.1	245	35.7	166	86
		65.0	12	43.3	252	34.6	169	74
		65.0	18	42.1	252	34.3	175	97
A	244	58.0	0	43.8	228	34.0	145	64[c]
		63.5	6	44.1	245	34.4	158	71
		65.0	12	43.3	252	33.4	160	64
		65.0	18	42.1	252	32.9	165	79

[a] Calculated from weight of ingredients.
[b] Not heat-treated.
[c] Total water in dough found in bread.

the bread from no-milk doughs baked at 210°C. was 77% for flour WW, 73% for flour B, and 72% for flour A (Table V). When commer-

cial, heat-treated, nonfat dry milk was included in the doughs, at all levels and at a baking temperature of 210°C., from 69 to 82% of the water absorbed by the milk was found in the bread. When extra water was added to the no-milk doughs, from 69 to 80% was recovered in the bread (flour B, Table III). Consequently, it appears that heat-treated milk does not appreciably alter the percentage of added water which is lost during baking.

At the higher baking temperatures, somewhat less water was retained by milk than by flour alone (flour B). For flour A, which had a lower water absorption than did the other flours, and which did not tolerate so high an absorption for the milk, there is some indication that a higher percentage of the water absorbed by the milk was retained in the bread than was retained by the flour, especially at the higher milk levels where the water absorption of the milk was increasingly lowered.

When 12% nonfat dry milk which was not heat-treated was added to flour B, 183, 248, and 262% of the water added to the dough with the milk was retained at baking temperatures of 210°, 221°, and 232°C., respectively. The water absorption of the milk was very low (0.08 g. water per g. milk), and it is apparent that the milk retained some of the flour water in the bread. Nevertheless, the moisture content of the breads containing the unheated milk was much lower than that of the breads containing no milk or the heat-treated milk.

(f) Loaf volume: The loaf volumes of bread made from flours WW, B, and A were increased an average of 60 cc. (range, 20 to 135 cc.) over those of the controls at all baking temperatures used when 6% nonfat dry milk (preheated or not) was incorporated into the doughs. Preheated nonfat dry milk used at the 12% level either did not change, or else increased (average increase, 25 cc.), the loaf volume with flours WW, B, and A, with one exception. The 12% milk loaf with flour A decreased 65 cc. when baked at 244°C. When given no preheated treatment, 12% nonfat dry milk decreased the loaf volume of bread made from flour B from 165 to 260 cc. at baking temperatures of 210°, 221°, and 232°C. In general, 18% nonfat dry milk decreased loaf volume except with flours WW and A baked at 210°C., in which slight increases were found.

Milk Fractions. The effects of the addition of calcium caseinate, of whey, of a partially delactosed nonfat dry milk, and of soluble milk albumin solids on water absorption, dough and bread yield, and proofing, baking, and cooling losses are shown in Table VI. Amounts of water in dough and in bread, and percentages of added water in dough found in bread are given in Table VII.

TABLE VI
EFFECT OF VARIOUS INGREDIENTS ON PROOFING, BAKING, AND COOLING LOSS AND ON BREAD YIELD
(Flour B – baking temperature 210°C.)

WATER ABSORPTION	ADDED INGREDIENT		ABSORPTION	WEIGHT OF DOUGH[b]	WEIGHT OF BREAD	INCREASE IN YIELD OVER CONTROL		PROOFING, BAKING, AND COOLING LOSS
	Kind	Amount[a]				Dough	Bread	
%		%	*g water / g ingredient*	g	g	%	%	%
63.5		...	...	516	443	...	...	13.3
79.0	Calcium caseinate	6	2.72	581	494	12.6	11.7	14.0
91.0		12	2.38	634	540	22.9	21.9	14.0
67.0	Whey	6	0.60	544	466	5.4	5.3	13.4
69.0		12	0.49	568	492	10.1	11.2	12.5
67.0	Soluble milk	3	1.25	535	464	3.7	4.6	13.5
69.0	albumin solids	6	1.02	551	478	6.8	8.0	12.3
73.0	Soluble milk	3	3.38	555	476	7.6	7.6	13.3
82.5	albumin solids,	6	3.33	592	510	14.7	15.2	13.0
101.5	heat-treated	12	3.28	666	580	29.1	31.0	12.1
67.5	Nonfat milk	6	0.72	546	469	5.8	6.0	13.1
69.0	solids, partially delactosed	12	0.51	568	495	10.1	11.8	12.0
69.0	Soy flour, full-fat	6	1.10	548	474	6.2	7.1	12.6
74.5		12	1.07	580	501	12.4	13.2	12.7
93.0	Soy flour and nonfat dry milk, heat-treated	6 6	1.07	584	503	13.2	13.6	13.1
72.5	Sweet rice flour	6	1.74	558	481	8.1	8.6	13.0
79.0		12	1.51	594	509	15.1	14.9	13.5
69.5	Potato flour	6	1.23	550	472	6.6	6.6	13.4
75.5		12	1.20	584	500	13.2	12.9	13.6
69.0	Corn starch	6	1.08	548	471	6.2	6.4	13.2
75.0		12	1.08	581	500	12.6	12.8	13.1
69.5	Wheat starch	6	1.09	549	474	6.4	7.0	12.9
75.0		12	1.09	582	501	12.8	13.1	13.0
66.0	Calcium peroxide	0.5	5.40	525	453	1.7	2.3	13.0

[a] Flour basis.
[b] Calculated from weight of ingredients.

(a) Calcium caseinate: The calcium caseinate used was not entirely soluble. It had a very high hydration capacity of over 2 g. of water per g. of ingredients, but during fermentation the doughs slackened and became very sticky.[2] This sample of calcium caseinate did not decrease baking loss. In fact, average proofing, baking, and cooling losses (14.0% for both 6 and 12% calcium caseinate) were significantly higher than for control doughs (13.3%). The water contents of the breads were high because of high moisture levels in the doughs. The water absorbed

[2] Pyenson and Dahle (18) found that casein had a bound-water content over twice that of nonfat milk solids.

TABLE VII

EFFECT OF VARIOUS INGREDIENTS ON THE MOISTURE CONTENT OF BREAD

(Flour B – baking temperature 210°C.)

WATER ABSORPTION	ADDED INGREDIENT		WATER IN DOUGH		WATER IN BREAD		ADDED WATER IN DOUGH FOUND IN BREAD
	Kind	Amount[a]					
%		%	%	g	%	g	%
63.5	.....	...	45.0	232	38.0	168	73[b]
79.0	Calcium caseinate	6	48.1	279	41.1	203	74
91.0		12	49.7	315	42.8	231	75
67.0	Whey	6	44.6	242	37.8	176	71
69.0		12	43.9	249	37.0	182	80
67.0	Soluble milk	3	45.3	243	38.8	180	110
69.0	albumin solids	6	45.4	250	39.0	187	101
73.0	Soluble milk	3	47.2	262	40.6	194	84
82.5	albumin solids,	6	49.0	290	43.0	220	87
101.5	heat-treated	12	52.1	347	47.1	273	91
67.5	Nonfat dry milk, par-	6	44.8	245	38.1	179	82
69.0	tially delactosed	12	43.9	250	37.7	186	101
69.0	Soy flour, full-fat	6	45.4	249	38.8	184	92
74.5		12	45.7	265	39.2	196	84
93.0	Soy flour and nonfat dry milk, heat-treated	6 6	45.8	268	39.0	196	78
72.5	Sweet rice flour	6	46.4	259	39.5	190	79
79.0		12	47.0	279	39.8	203	73
69.5	Potato flour	6	45.6	251	38.6	182	73
75.5		12	46.1	269	38.8	194	68
69.0	Corn starch	6	45.4	249	38.5	181	77
75.0		12	45.8	266	39.0	195	78
69.5	Wheat starch	6	45.5	250	38.7	183	83
75.0		12	45.9	267	39.1	196	79
66.0	Calcium peroxide	0.5	45.7	240	39.1	177	104

[a] Flour basis.
[b] Total water in dough found in bread.

by the casein was baked out in about the same quantity as the water in the control dough, since the added water in the dough found in the bread was similar for both control and casein doughs. In the present work, 6% casein increased the loaf volume of 495-g. doughs by 75 cc., while the volume of 12% casein loaves (2,160 cc.) was similar to that of the control (2,170 cc.). Gordon *et al.* (10) reported that casein lowered the loaf volumes of bread made without shortening.

(b) Whey: The water absorption of this sample of whey, which had received no heat treatment, was low. Dough and bread yields were increased, but not so much as with dough ingredients of higher absorption. Proofing, baking, and cooling losses were decreased with 12% whey, but the breads were less moist than the control loaves. The added

dough water found in the bread was within the range of that found by varying the water in the control doughs. Loaf volume was increased 170 cc. by 6% whey, but decreased 230 cc. by 12% whey. Kuhlmann and Golossowa (14) reported that whey increased the water-binding capacity of dough.

(c) Soluble milk albumin solids: The water absorption of the albumin solids was high, and was increased by heat treatment to over 3 g. of water per g. of solids. The addition of heat-treated soluble milk albumin solids gave the largest increases in dough and bread yields of any of the ingredients tried. Since the proofing, baking, and cooling losses were the same as, or lower than, those of the control loaves, the moisture in the bread was high, particularly with the heat-treated solids. A higher percentage of water (84 to 110%) added with the albumin solids was found in the bread than was found by adding water to the control doughs. This retention of water is not surprising in view of the high protein content (83%) and water-binding capacity of albumin. Pyenson and Dahle (18) reported that the albumin present in milk is about twice as hydrophilic as casein. Loaf volumes were decreased by the albumin solids: 70 and 130 cc. for 3 and 6% of the nonheated sample, and 155, 455, and 1,025 cc. for 3, 6, and 12% of the heat-treated solids. Loaves containing 6 and 12% of the heat-treated albumin solids were heavy and compact. Twelve percent levels of the nonheat-treated albumin were not used, because of their softening action on the dough, which caused extreme stickiness.

(d) Partially delactosed nonfat dry milk: The effect of this product, which had received no heat treatment, was somewhat similar to that of the unheated, nonfat dry milk although the water absorption and, consequently, dough and bread yields and water in bread were higher.

Flours, Starches, and Calcium Peroxide. The effects of added, full-fat soy flour, sweet rice flour, potato flour, corn starch, wheat starch, and calcium peroxide on water absorption, bread yield, and proofing, baking, and cooling losses are shown in Table VI. The effects on water in dough and in bread are given in Table VII. Calcium peroxide and sweet rice flour had the highest water absorptions (5.40 and 1.5–1.7 g. water per g. ingredient, respectively), which are reflected in the higher dough and bread yields. Potato flour and soy flour had greater absorptions (1.2 and 1.1 g. water per g. ingredient) than did the starches and wheat flour. Some of these trends in water absorption have been found by other workers. Harris *et al.* (12) stated that cooked potato flour generally raised the flour absorption, and Ofelt *et al.* (16) found that full-fat soy flour also increased the water absorption of the dough. Kuhlmann and Golossowa (14) found the water-binding capacity of

soya flour to be highest of any of the flours tested, but that of potato flour, the lowest.

Bread yields were greater than dough yields when proofing, baking, and cooling losses were lowered. This is particularly apparent with soy flour and somewhat less so with calcium peroxide and wheat starch.

All of these ingredients gave bread with a moisture content greater than that of the control. In all cases the water absorption used for the ingredients was greater than for the flour and, except for potato flour and 12% rice flour, proofing, baking, and cooling losses were slightly less than in the control loaves. The highest bread moistures were obtained with 6 and 12% sweet rice flour, 12% soy flour, wheat starch, and calcium peroxide, all of which increased the moisture 1% or more as compared with the control. Previously, Harris *et al.* (12) reported that cooked potato flour apparently increased crumb hydration (g. water per g. crumb) but did not materially increase moisture retention in 72 hours. Sieburth *et al.* (22) found that a potato product increased the moisture content of bread under some conditions but not under others. Bayfield and Swanson (5) stated that soy flour reduced the fermentation and baking loss in proportion to the amount added.

From 68 to 84% of the water added to the dough with the experimental ingredient was found in the bread in all cases except with 6% soy flour (92%) and calcium peroxide (104%). The latter two retained a higher percentage of the added water in the bread than was found in control loaves with added water (69 to 80%, Table III).

Calcium peroxide and all of the flours and starches tried, except for potato flour, decreased loaf volume by from 100 to 200 cc., the decrease being greater with the larger amount of ingredient. The volume of the control loaves averaged 2,170 cc. Both 6 and 12% potato flour increased loaf volume by about 50 cc. Harris *et al.* (12) reported that 1 and 4% cooked potato flour tended to reduce loaf volume with a malt-phosphate formula, but improved it with a lean formula. Sieburth *et al.* (22) found that, when substituted for white or whole-wheat flour, a potato product decreased loaf volume slightly. When the potato product was added to the flour with an equivalent amount of water, there was little change in volume. Soy flour has been found to give loaf volume nearly equivalent to that of the basic loaf when potassium bromate was used in the range of 1.0 to 3.0 mg.% on wheat-flour basis (16).

Commercial Bread. Results of a study of some commercial bakery practices are given in Table VIII. In bakery A, in the cases where there were four samples of bread, the doughs had been individually scaled to 17 oz., tagged, and weighed when 1 hour out of the oven, so that accurate baking losses were determined. Since the scalers were not exact,

TABLE VIII

STUDY OF COMMERCIAL BAKERY PRACTICES

Kind of Bread	Added Ingredients	Flour Weight Basis	No. of Loaves	Absorption[a]	Baking Time and Average Temperature		Scaled Dough Weight	Average Bread Weight	Average Baking and Cooling Loss	Water in Bread[b]	Bread	
											Loaf Volume	Specific Volume
		%		%	minutes	°F	oz	oz	%	%	cc	cc/g
Bakery A												
White	Nonfat dry milk	3	4	60	24	400	17.0	15.0	11.8	38.3	2543	5.98
White	Nonfat dry milk	5	1	60	40	400	=25.0	22.4	=10.4	36.8	3397	5.35
"Wheat"	Whole-wheat flour	20[c]	4	60	24	400	17.0	15.0	11.5	36.9	2320	5.44
100% Whole-wheat	.....	...	4	58	25	400	17.0	15.0	11.6	39.0	2018	4.74
Fresh mill 100% whole-wheat	.....	...	1	67	40	400	=25.0	23.4	= 6.4	38.5	1706	2.57
Cracked wheat	Cracked wheat	34[c]	1	63	25	400	=17.0	15.3	=10.2	37.4	1606	3.71
Potato	Potato culture	4	1	62	25	400	=17.0	15.0	=12.3	37.6	2731	6.44
Soya	Soy flour	10	1	57	25	400	=17.0	15.3	=10.1	36.3	2267	5.23
Milk and soy flour blend	Nonfat dry milk, soy flour	8 6	4	69	25	400	17.0	14.9	12.5	38.9 ± 0.2	2065	4.90
Rye	Rye flour	20[c]	1	63	25	400	=17.0	15.6	= 8.5	37.1	1807	4.10
Raisin	Raisins	65	4	65	25	400	17.0	15.6	8.2	36.0	1817	4.11
Bakery B												
White	Nonfat dry milk	1	1	58	24	510	=17.75	15.9	=10.2	36.0		
Buttermilk	Buttermilk solids	8½	1	60	24	490	=17.75	16.5	= 7.1	36.9		
"Wheat"	Whole-wheat flour	35[c]	1	56	24	500	=17.50	15.8	= 9.4	36.7		
100% Whole-wheat	.....	...	2	60	24	500	=17.75	15.4	=13.2	37.6 ± 0.1		

[a] As-is flour basis.
[b] Determinations on one loaf, except on two where indicated.
[c] Part of flour.

baking losses of the other loaves from bakery A and those from bakery B were only approximate.

There were considerable differences in the practices of these two bakeries. Bakery A scaled at lower dough weights, baked at lower oven temperatures, and the baked loaves were closer to the minimum weight for 15-oz. loaves. The moisture content of some of the loaves exceeded the federal standard of 38%.

The question is often raised, "When is a loaf of bread baked?" Modern commercial baking methods vary considerably from practices of the past. In 1910, Atwater (2) stated, "For ordinary purposes, a baker heats the oven to 400° or 500°F. (204° or 260°C.) and lets a pound loaf bake an hour or an hour and a quarter." Darnell (8), in 1937, in a survey discussion of commercial oven types, found that all ovens required 28 or more minutes for baking 1-lb. loaves; on the "flat" oven, a 1-lb. loaf could be baked properly, with heat from 480° to 510°F. (249° to 266°C.) in from 28 to 32 minutes. Stout and Drosten (26), in 1933, stated that boiling temperature in the interior of the dough "must have been maintained for at least 9 minutes to produce a done loaf of bread."

In connection with the latter statement, an experiment was tried to determine how rapidly the interior of doughs baked at 210° and 244°C. attained boiling temperature. Thermometers were inserted in the doughs before proofing, and the temperature rise was watched closely during the baking period. Results are given in Table IX. Since boiling

TABLE IX

EFFECT OF BAKING TEMPERATURE ON TIME DOUGH INTERIOR IS MAINTAINED AT 100°C.

Scaled Dough Weight	Baking Time	Baking Temperature	Time at 100° C.	Bread Weight	Baking and Cooling Loss	Water in Bread
g	*minutes*	*°C*	*minutes*	*g*	%	%
488.0	30	210	6	427.2	12.5	38.0
488.0	30	244	9	408.4	16.4	35.9
			Bakery A			
483.0	24	204	?	425.9	11.8	38.3

temperature in the loaf baked at 210°C. was maintained for only the last 6 minutes of the baking period, it is probable that white bread from bakery A, baked a shorter period of time at 400°F. (204°C.), was underbaked.

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Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minnesota (1957).
2. Atwater, H. W. Bread and breadmaking. U. S. Dept. Agr., Farmers' Bull. 389. Government Printing Office: Washington, D.C. (1910).
3. Baker, J. C., Parker, H. K., and Mize, M. D. The distribution of water in dough. Cereal Chem. **23:** 30–38 (1946).
4. Barackman, R. A., and Bell, R. N. Temperatures attained in baking products. Cereal Chem. **15:** 841–845 (1938).
5. Bayfield, E. G., and Swanson, E. C. Effect of yeast, bromate, and fermentation on bread containing soy flour. Cereal Chem. **23:** 104–113 (1946).
6. Cathcart, W. H. Moisture content of bread and its comparison with other staple foods in the diet. Proc. Inst. Food Technologists, pp. 51–58 (1940).
7. Choi, R. P. Dry milk. Trans. Am. Assoc. Cereal Chemists **10:** 113–119 (1952).
8. Darnell, G. Oven temperatures. Proc. 14th Ann. Meeting, Am. Soc. Bakery Engrs., pp. 39–40 (1937).
9. Garnatz, G., and Kornreich, E. G. The effects of absorption in the breadmaking process and resultant bread under commercial shop conditions. Proc. 19th Ann. Meeting, Am. Soc. Bakery Engrs., pp. 42–48 (1942).
10. Gordon, A. L., Jenness, R., and Geddes, W. F. The baking behavior of casein and whey prepared from skimmilk by various procedures. Cereal Chem. **31:** 1–6 (1954).
11. Halton, P. Relation of water absorption to the physical properties and baking quality of flour doughs. Cereal Chem. **15:** 282–294 (1938).
12. Harris, R. H., Sibbitt, L. D., and Banasik, O. J. Effect of potato flour on bread quality and changes with age. Cereal Chem. **29:** 123–131 (1952).
13. Kennedy, Barbara M., Fletcher, Loraine R., and Sabiston, Adelia R. Studies on the incorporation of nonfat milk solids in whole wheat bread. I. Dough properties and baking tests. Cereal Chem. **31:** 347–361 (1954).
14. Kuhlmann, A. G., and Golossowa, O. N. Bound water in bread making. Cereal Chem. **13:** 202–217 (1936).
15. Micka, J., and Child, Elizabeth. Some factors influencing absorption in experimental baking. Cereal Chem. **5:** 208–214 (1928).
16. Offelt, C. W., Smith, A. K., and Derges, R. E. Baking behavior and oxidation requirements of soy flour. I. Commercial full-fat soy flours. Cereal Chem. **31:** 15–22 (1954).
17. Platt, W. Method for making super milk bread commercially. Proc. 12th Ann. Meeting, Am. Soc. Bakery Engrs., pp. 79–82 (1935).
18. Pyenson, H., and Dahle, C. D. Bound water and its relation to some dairy products. I. The bound water content of some milk products and other products used in the dairy industry. J. Dairy Sci. **21:** 169–185 (1938).
19. St. John, J. L., and Bailey, C. H. The effect of dry skimmilk upon the water absorption of doughs and the plasticity of flour suspensions. Cereal Chem. **6:** 140–150 (1929).
20. Shipstead, J. Methods of testing for absorption value of nonfat dry milk solids in bread dough. J. Dairy Sci. **34:** 1092–1103 (1951).
21. Sieber, W. A. An analysis of white breads and related observations. Am. J. Pharm. **114:** 422–437 (1942).
22. Sieburth, Janice F., Wahl, T., and McLaren, Barbara A. Effect of added softeners on moisture content of bread. J. Am. Dietet. Assoc. **30:** 355–357 (1954).
23. Skovholt, O., and Bailey, C. H. Free and bound water in bread doughs. Cereal Chem. **12:** 321–355 (1935).
24. Skovholt, O., and Garnatz, G. Water in the baking process. Proc. 18th Ann. Meeting, Am. Soc. Bakery Engrs., pp. 23–32 (1941).
25. Stamberg, O. E. Bread baking quality of dry milk solids. The Bakers Digest **20:** 30–31 (1946).
26. Stout, L. E., and Drosten, F. Heat flow through bakery products. I. Time-temperature relationships existing during the baking of bread. Ind. Eng. Chem. **25:** 428–430 (1933).

27. UNITED STATES GOVERNMENT. Bakery products: definitions and standards of identity. Federal Register **17**(96): 4453–4464 (May 15, 1952).
28. VAIL, GLADYS E., and BAILEY, C. H. The state of water in colloidal gels: Free and bound water in bread doughs. Cereal Chem. **17**: 397–417 (1940).
29. WALKER, N. H. Report on moisture in raisin, fruit, and white bread. J. Assoc. Offic. Agr. Chemists **30**: 249–251 (1947).

EFFECT OF OXIDIZED POLYSACCHARIDES ON DOUGH AND BAKING PROPERTIES OF WHEAT FLOUR[1]

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ABSTRACT

Oxidized polysaccharides obtained by periodate oxidation showed marked improving action on dough and on baking characteristics of wheat flour. Periodate-oxidized polysaccharides added to wheat flour increased the resistance to extension and decreased the extensibility of nonyeasted dough as measured in the Extensograph®. Oxidized locust-bean gum (10–100% oxidized) and wheat starch (100% oxidized) were investigated. The latter produced an optimal improving effect at concentration levels of 0.03–0.1%. It is assumed that the improving effect is caused by a reaction of the aldehyde groups of the oxidized polysaccharides with flour proteins.

Of the various theories advanced for the explanation of the action of flour improvers (e.g. potassium bromate) the one which assumes that oxidation takes place at the sulfhydryl groups of flour proteins is probably still the most popular (8). It has been shown recently, however, that in addition to the protein fraction, the flour lipids seem also to be involved in the action of oxidative improvers (2). Furthermore, Rotsch (7) reported in 1954 that "synthetic," protein-free doughs consisting of wheat starch and a cold water-swelling polysaccharide (e.g. locust bean gum) also seemed to respond to potassium bromate. It might be assumed, therefore, that the carbohydrate components of flour are associated with the improving effect of oxidizing agents.

By treating polysaccharides with specific oxidizing agents, reactive groups (e.g. aldehydes) can be introduced into the polysaccharide molecule, which in turn may react with flour proteins, forming a three-dimensional network. Under certain conditions this may lead

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to an increase in toughness and rigidity of the dough. It should be possible to transform starch into an oxidized reactive form which, incorporated into flour, would act as an improver by virtue of its polyfunctionality.

One of the most specific oxidizing agents for polysaccharides is periodic acid and its salts which oxidize hydroxyl groups on adjacent carbon atoms to dialdehydes (3). The reaction product is a polyaldehyde possessing a number of reactive aldehyde groups depending on the degree of oxidation. By using more or less periodate for the oxidation of the polysaccharides, the degree of oxidation (aldehyde content) can be easily varied.

Since these polysaccharides could act as flour improvers it seemed of interest to study their effects on dough and baking characteristics of wheat flour.

Most of the work described in this paper has been carried out with periodate-oxidized wheat starch and locust bean gum.

Materials and Methods

Materials. Wheat starch and locust bean gum were commercial food grade products. The locust bean gum was purified by solution in water, filtration with Celite, and precipitation with alcohol.

The flours used were: No. 1, a commercial unbleached white bread flour and No. 2, a commercial low-grade white bread flour from domestic wheat. Both flours were untreated.

Preparation of Oxidized Wheat Starch. Aqueous slurries of wheat starch (about 5%) containing the theoretical amounts of sodium-meta periodate to oxidize all glucose residues to the dialdehyde (1 mole of sodium-meta periodate per glucose unit of starch) were shaken in the dark for 48 hours at room temperature. The pH was adjusted to 4.7 by addition of sodium acetate-acetic acid buffer. The oxidized starch was recovered by filtration and washed carefully with water until free of iodate. This step is important because iodate itself is a powerful flour improver. Removal of iodate was checked by adding potassium iodide solution and acetic acid to the oxidized starch. The periodate-oxidized starch was dried at room temperature.

Preparation of Oxidized Locust Bean Gum. To 1% solutions of purified locust bean gum the theoretical amounts of sodium-meta periodate were added to obtain respectively 10, 25, 50, 75, and 100% degrees of oxidation of the polysaccharide. When necessary, the pH was adjusted to 4.0 with acetic acid. The oxidations were allowed to proceed in the dark for 20 hours at about 15°C. Tests for residual periodate were all negative after this reaction time. The oxidation

products were recovered by precipitation in two volumes of ethanol. The iodate was removed by washing 12 to 15 times with 50% aqueous ethanol until tests for iodate were negative. The products were finally washed with ethanol (96%) and ether and dried *in vacuo* at 40°C.

Testing of Dough Properties. The effect of the various oxidized polysaccharides on the dough properties of flours 1 and 2 was determined with a Brabender Extensograph® using the standard technique. Absorption was adjusted to 500 Brabender Units after 1 minute's mixing time. At the end of a 5-minute resting period the mixer was run for 2 more minutes. If necessary a little water was added (not more than 0.5%) to maintain the consistency of 500 units. The polysaccharides were dissolved by heating in part of the water used to prepare the doughs; 6 g. of sodium chloride were added to this solution before addition to the mixing bowl. Curves were drawn for duplicate doughs at 45, 90, and 135 minutes. Only the latter are shown in this paper. The length of the curves (extensibility), the maximum height (resistance), and the area under the curves were measured and compared.

Baking Tests. A few preliminary baking tests were carried out with the same flour (No. 2), to which were added the same amounts of 100% oxidized wheat starch as were used in the extensograms in Fig. 2. The baking tests were carried out at the Swiss Bakery School and Experiment Station in Lucerne. Pan breads and round loaves (free-standing) were baked from batches of 1 kg. of flour using 25 g. of sodium chloride and 30 g. of compressed yeast. No malt was added. The loaf volumes of the pan and round breads were measured. In addition, the height and the mean diameter of the round loaves were determined. The ratio height/diameter (h/d) gives a measure of the form of the loaf. Flat breads give lower values than taller breads.

Results and Discussion

The Extensograms in Figs. 1 and 2 clearly show that the oxidized polysaccharides at a certain concentration level had a distinct improving effect on the dough properties. The extensibility and resistance of the doughs could be manipulated to give values which, from experience, are considered to be brought about by flour improvers. It is therefore highly probable that these modified polysaccharides react with flour constituents. That the improving effect is caused by the aldehyde groups introduced into the polysaccharides is evident from Fig. 1, which shows that increasing the degree of oxidation enhances the improving effect. The Extensograms in Fig. 2 demonstrate that optimal improvement with 100% oxidized wheat starch was ob-

tained with amounts of 0.03–0.05% based on the weight of flour. This is roughly 10 to 15 times the amount required with conventional flour improvers (potassium bromate or ascorbic acid). Overtreatment resulted in very short and tough doughs (e.g. Fig. 2, G).

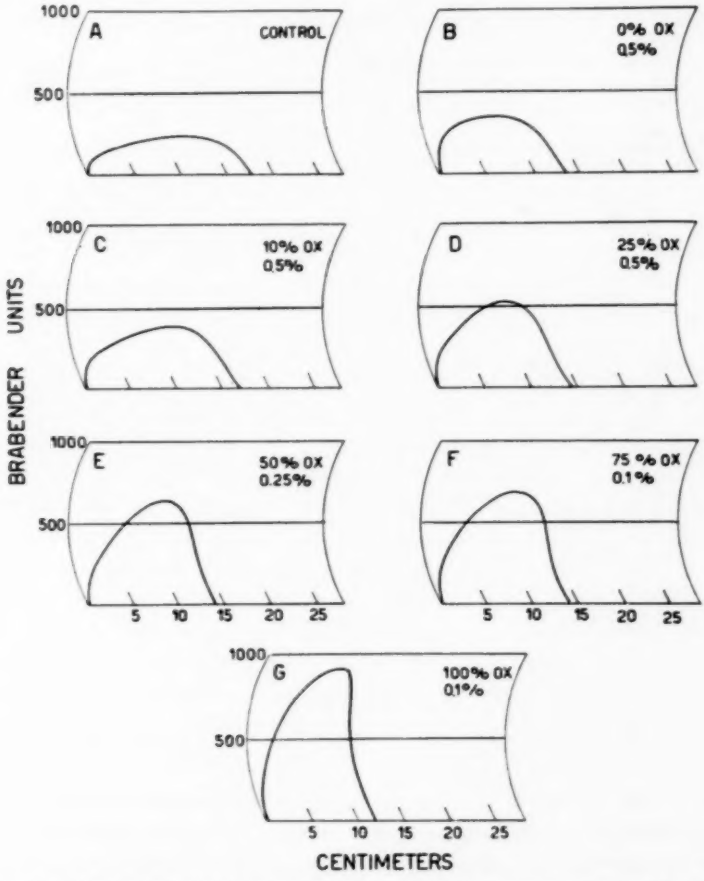

Fig. 1. Effect of oxidized locust bean gum on the Extensogram curves of flour (1). The figures in the upper right-hand corners give percentage of oxidation of locust bean gum and the amounts added based on the flour weight.

Baking tests on mixtures of flour No. 2 with small percentages of oxidized wheat starch showed that these additions caused a positive response (Table I). The improvement was more evident with the round breads, which were less flat and had larger volumes than the untreated loaf. The round loaf baked with 0.03% oxidized starch had the highest score with respect to shape and appearance. The largest

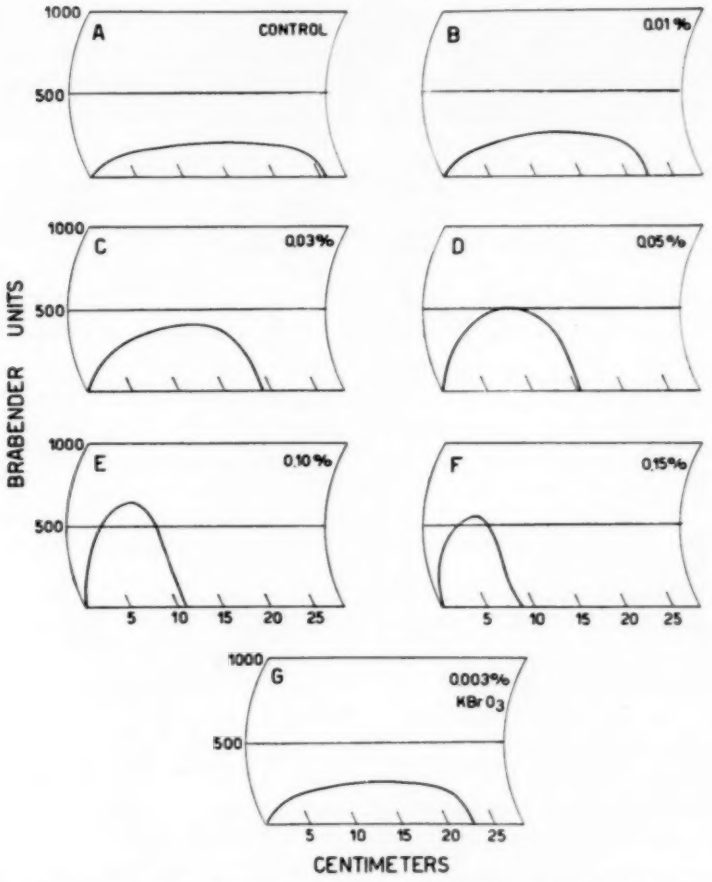

Fig. 2. Effect of various amounts of 100% oxidized wheat starch on extensogram curves of flour (2).

volume with pan bread was obtained when 0.1% of the oxidized starch was added. The observed increase in bread volume was lower compared to the volume increase obtained with potassium bromate, in spite of the superior Extensogram curves obtained with the oxidized starch. Overtreatment (0.15%) resulted in very short doughs giving breads with significantly smaller volumes. It is particularly noteworthy that higher levels of oxidized polysaccharides (>0.1%) resulted in increasing brownness of the bread crumb, probably caused by a Maillard type reaction of the oxidized polysaccharides with the flour proteins. Similar observations with baking tests were also made with oxidized locust bean gum. Oxidized locust bean gum and oxidized dextrins likewise produced a brown crumb at higher concentrations.

TABLE I

EFFECT OF VARIOUS AMOUNTS OF 100% OXIDIZED WHEAT STARCH ON LOAF VOLUME AND FORM

OXIDIZED WHEAT STARCH ADDED	PAN BREAD VOLUME[a]	ROUND BREAD VOLUME[b]	H/D × 100
%	*ml.*	*ml.*	
Control	511	1020	572
0.01	524	1070	559
0.03	519	1020	628
0.05	545	1070	621
0.1	558	870	535
0.15	489	820	515
0.003 Potassium bromate	584	1070	659

[a] From 100 g. of flour.
[b] From 300 g. of dough.

The observed effects of oxidized polysaccharides on dough can best be explained by assuming that the aldehyde groups of the modified polysaccharides react with the flour proteins probably through their amino groups. By this reaction cross-links are formed between the oxidized polysaccharides and the proteins. Formation of such a loose three-dimensional network would explain the modified physical properties of the doughs, e.g., increased resistance and toughness. The observation that slight overtreatment produces short and tough doughs with resulting smaller bread volume suggests that there exists an optimal degree of cross-linking for production of optimal dough properties. Cross-linking of proteins by simple aldehydes like formaldehyde and glyoxal is well known (4). Hlynka and Bass (5) have theorized that simple carbohydrates through their reducing groups may act as cross-linking agents between protein molecules. The described oxidized polysaccharides have the advantage that they are polyfunctional aldehydes of high molecular weight which may be obtained by proper modification of natural flour constituents, e.g., wheat starch.

How far the observed effects of the oxidized wheat starch might be suggestive of the mode of action of improvers in general cannot be stated at present. Most of the conventional improvers (e.g., potassium bromate, sodium chlorite, and particularly sodium iodate) are very poor oxidizing agents for polysaccharides. Nevertheless, they act as improvers at considerably lower concentration levels than the investigated oxidized polysaccharides. It is therefore unlikely that these improvers act via the oxidation of wheat starch. Whether the soluble flour pentosans, which are known to be much more sensitive towards oxidizing agents than starch (1), are involved in the reaction mechanism of improvers has not been established so far.[2] Udy (9) has

[2] The action of various oxidizing agents on flour pentosans will be discussed in a subsequent paper.

shown recently that the presence of gluten proteins greatly accelerates the gelification of pentosan solutions by potassium bromate. This might be caused by the reaction of oxidized pentosans with gluten proteins.

These limited experiments on the effect of oxidized polysaccharides on dough and baking properties should be extended to other types of flours in order to establish fully and to evaluate their improving effects. Since improved methods for the preparation of periodate-oxidized starch have been recently published (6), their production should be commercially feasible.

Acknowledgments

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Literature Cited

1. Baker, J. C., Parker, H. K., and Mize, M. D. The pentosans of wheat flour. Cereal Chem. **20:** 267–280 (1943).
2. Cookson, M. A., and Coppock, J. B. M. The role of lipids in baking. III. Some breadmaking and other properties of defatted flours and flour lipids. J. Sci. Food Agr. **5:** 72–87 (1956).
3. Dyer, J. R. Use of periodate oxidations in biochemical analysis. *In* Methods of biochemical analysis, ed. by D. Glick, Vol. III, pp. 111–153. Interscience: New York (1956).
4. French, D., and Edsall, J. T. The reactions of formaldehyde with amino acids and proteins. *In* Advances in protein chemistry, ed. by M. L. Anson and J. T. Edsall, Vol. II, pp. 278–333. Academic Press: New York (1945).
5. Hlynka, I., and Bass, E. J. Reaction of dough and gluten with glucose. Cereal Chem. **26:** 513–518 (1949).
6. Mehltretter, C. L., Rankin, J. C., and Watson, P. R. Electrolytic preparation of periodate oxystarch. Ind. Eng. Chem. **49:** 350–354 (1957).
7. Rotsch, A. Chemische und backtechnische Untersuchungen an künstlichen Teigen. Brot und Gebäck **8:** 129–130 (1954).
8. Sullivan, Betty. Proteins in flour J. Agr. Food Chem. **2:** 1231–1234 (1954).
9. Udy, D. C. Interactions between proteins and polysaccharides of wheat flour. Cereal Chem. **34:** 37–46 (1957).

STUDIES ON CORN PROTEINS

IV. Protein and Amino Acid Content of Different Corn Varieties[1]

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ABSTRACT

No major differences in amino acid composition were observed between several U.S. and Guatemalan corn (*Zea mays*) selections. The percent of total nitrogen of the grain contributed by the pericarp plus tip cap varied from 1.8 in U.S. High Protein (HP) to 3.3 in U.S. 4251; the percent contributed by the endosperm varied from 68.4 in U.S. High Oil (HO) to 83.2 in U.S. High Protein (H5); the percent contributed by the germ varied from 14.6 in U.S. High Protein (H5) to 29.2 in U.S. High Oil (HO). The Guatemalan selections had intermediate values.

The proteins of the germ and endosperm of the U.S. and Guatemalan selections were extracted with an alkaline copper reagent and the extracts separated into three fractions by a method described previously. In the endosperm samples, the acid-soluble proteins varied from 17 to 26%, the alkali- and alcohol-soluble proteins (zein) from 41 to 60%, and the alkali-soluble, alcohol-insoluble proteins (glutelins) from 17 to 31% of the total nitrogen. Similar treatment of the corn germs gave 30–40% acid-soluble, 5–10% alkali- and alcohol-soluble, and 49–54% alkali-soluble, alcohol-insoluble proteins as a percent of the total nitrogen. The copper extraction-fractionation method should prove useful to the corn breeder in the development of new types of corn.

The preceding papers in this series (10, 11, 12) describe a method for the nearly quantitative extraction of proteins from corn germ and endosperm, and the separation of the extracts into three fractions, namely, (a) acid-soluble, (b) alkali- and alcohol-soluble, and (c) alkali-soluble, alcohol-insoluble. In addition, data on the nature of the proteins in fractions (a) and (c) of endosperm have been presented (10, 12). These special techniques of extraction and fractionation have served as a new method of analysis of corn, which has supplied useful data on the protein and amino acid make-up of several varieties selected for study.

Materials and Methods

Corn Varieties Selected. The twelve varieties selected for analysis are listed in Table I. U.S. 4251 is a yellow commercial Midwest hybrid; U.S. High Oil HO a white high-oil (large-germ) single cross; U. S. High Protein H5 a yellow, high-protein single cross, and U.S. High

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Protein HP a white, high-protein hybrid. The U.S. mutant, shrunken-floury (*sh*^{fl}), is a modified flint line which lacks the horny endosperm (13).

TABLE I
CORN SELECTIONS

United States		Guatemala	
Corn No.	Name	Corn No.	Name
1	4251	7	Tiguisate (TGY)
2	High Oil (HO)	8	San Sebastian (SSD)
3	High Protein (H5)	9	142–48
4	High Protein (HP)	10	Cuyuta
5	Normal *Sh*^{fl}	11	Teosinte
6	Mutant *sh*^{fl}	12	Teosinte × Corn

The four varieties of Guatemalan corn (Table I) represent corns cultivated in the low and high land of that country, and are in general representative of the corns consumed in the form of tortillas. Three of the corns, Tiguisate (TGY), Guatemalan 142–48, and Cuyuta, cultivated in the lowlands, are open-pollinated flint-type varieties reaching maturity in 90 to 100 days. The San Sebastian (SSD) is a corn grown in the highlands and is an open-pollinated, starchy-type corn reaching maturity in 9 to 11 months. The Tiguisate (TGY) and Guatemalan 142–48 are yellow corns with high carotene content (5), whereas the San Sebastian (SSD) and Cuyuta are white corns with a low carotene content. Teosinte, a close relative of corn, was part of a 1953 crop cultivated experimentally in the Guatemalan lowlands. Corn × teosinte seed represents a natural cross of teosinte and lowland corn harvested in 1954 from the Guatemalan lowlands.

Methods of Analysis. The corn samples, except the teosinte and the corn × teosinte cross, were dissected by hand with a knife and pincers. Random samples of 20 seeds of grain replicated 20 times were weighed and placed in a dish with enough distilled water to cover the kernels. This was then placed for 1–2 hours in a refrigerator to soften the pericarp and tip cap, which came off easily with the aid of pincers. A knife was used to cut out the germ from the endosperm. Each batch consisted of the anatomical section from 20 seeds, and was air-dried for 3 days and then weighed. The teosinte was dissected with a pair of pliers, since the hull (rachis envelope; see ref. 17) is hard and does not soften easily. The anatomical sections obtained were the hull, the pericarp, and the germ plus endosperm. No attempt was made to separate the germ from the endosperm, since the germ is fairly small. The cross of corn × teosinte was not dissected because the sample weighed only 10 g. All samples were air-dried and ground to a particle

size of 0.001 in. in a burr mill. Low-lipid samples were obtained by extraction of ground samples in a Soxhlet for 36 hours with hexane (b.p. 65–67°).

Nitrogen analysis was carried out on the whole ground kernel, and on the ground anatomical sections of air-dried samples by the micro-Kjeldahl method (3). Amino acid assay of the whole kernels and of some of the isolated protein fractions was carried out according to the method of Steele *et al.* (16) using *Leuconostoc mesenteroides* and *L. citrovorum*. The copper extraction method used on the separated corn germ and endosperm is that described by Mertz and Bressani (11). The germ or endosperm extract plus "washing" (11) was then fractionated according to the method described by Mertz, Lloyd, and Bressani (12).

Results

Nitrogen Distribution in the Endosperm. Table II shows the distribution of the nitrogen of the endosperm among the one insoluble

TABLE II

NITROGEN DISTRIBUTION IN THE ENDOSPERM

CORN VARIETY	WHOLE-GRAIN PROTEIN	ENDOSPERM PROTEIN	RELATIVE PROPORTIONS OF VARIOUS FRACTIONS			
			Insoluble Nitrogen	Acid-Soluble Nitrogen	Alkali-Soluble Nitrogen	Alcohol-Soluble Nitrogen
	%	%	%	%	%	%
Guatemalan Cuyuta	8.0	7.4	10.2	25.5	23.1	41.2
U. S. 4251	8.2	7.2	1.9	26.3	28.0	43.8
San Sebastian (SSD) (Guat.)	8.6	7.9	10.9	20.8	17.6	50.6
Tiguisate (TGY) (Guat.)	9.8	9.4	8.6	23.4	22.3	45.7
Guatemalan 142–48	11.4	12.0	10.6	19.4	17.2	52.1
U.S. High Oil HO	12.4	12.7	11.0	16.6	23.9	48.5
U.S. Normal-*Sh*[1]	13.4	13.5	6.7	18.4	31.4	43.5
U.S. Mutant-*sh*[1]	13.8	12.3	6.0	21.5	25.9	46.6
U.S. High Protein H5	14.0	14.6	6.8	16.6	21.2	55.4
U.S. High Protein HP	18.2	18.2	2.6	18.0	19.7	59.7
Guatemalan teosinte × corn	21.0	...	2.9	20.2	23.3	53.6
Guatemalan teosinte	11.3	23.8[a]	4.3	18.9	21.3	55.5

[a] Dehulled. Both the teosinte and teosinte × corn samples contained the small germ.

and three soluble fractions obtained by the copper extraction-fractionation method. The corn varieties are listed in the order of increasing total crude protein contents (N × 6.25).

The insoluble nitrogen varied from 1.9 to 11.0% of the total nitrogen. This variation in completeness of extraction may be related to the distribution or nature of the nitrogen in the endosperm. The Guatemalan endosperms in general contained more insoluble nitrogen. The nature of the insoluble nitrogen is not known, and requires further investigation.

The acid-soluble nitrogen varied from 16.6% in the U.S. High Oil HO and U.S. High Protein H5 to 26.3% in U.S. 4251. In general, the acid-soluble nitrogen of the endosperm decreased with increasing crude protein content of the whole grain. The alkali-soluble, alcohol-insoluble (glutelin) nitrogen varied from 17.2% in Guatemalan 142–48 to 31.4% in U.S. normal–*sh*^{fl}. No clear-cut relationship is apparent between the percent of nitrogen in this fraction and the total protein of the grain.

The alcohol-soluble (zein) nitrogen varied from 41.2% in Guatemalan Cuyuta to 59.7% in U.S. High Protein HP. In general, the alcohol-soluble nitrogen of the endosperm increased with increasing crude protein content of the whole grain. This relationship has been observed by several workers (6, 7, 8, 9, 15).

With increasing crude protein content of the whole grain, the alcohol-soluble fraction of the endosperm in most cases increased at the expense of the acid-soluble fraction, and often also at the expense of the alkali-soluble (glutelin) fraction. To obtain more information on the relationship between the acid-soluble fraction and the zein and glutelin fractions, a study of the changes in proportions of these three fractions as corn grain matures is in progress.

The new extraction-fractionation method has proved useful in the evaluation of U.S. *sh*^{fl}-mutant, Guatemalan teosinte, and a Guatemalan teosinte cross with corn. In the case of the mutant, the question was raised whether corn lacking the horny endosperm would have a protein composition (in the endosperm) different from that of normal corn. The data in Table II show that there is only a small difference in the zein (alcohol-soluble nitrogen) content of the U.S. *Sh*^{fl}-normal and U.S. *sh*^{fl}-mutant endosperm, and small compensating differences in the other two soluble fractions. This suggests that the proteins of the floury endosperm do not differ markedly from those of the horny endosperm.

The possibility of improving the protein quality of corn by crossing it with teosinte does not appear promising on the basis of the analysis of teosinte and the teosinte-corn cross (Table II). Teosinte has about the same proportions of the three soluble fractions as U.S. High Protein HP and H5, two U.S. high protein corns containing high levels of zein. The cross of teosinte and Guatemalan corn produced a grain containing more zein (alcohol-soluble nitrogen) than any of the Guatemalan corns studied.

Nitrogen Distribution in the Germ. Table III shows the distribution of nitrogen in the germ of three U.S. and three Guatemalan corn

TABLE III

NITROGEN DISTRIBUTION IN THE GERM

CORN VARIETY	WHOLE-GRAIN PROTEIN	GERM PROTEIN	RELATIVE PROPORTIONS OF VARIOUS FRACTIONS			
			Insoluble Nitrogen	Acid-Soluble Nitrogen	Alkali-Soluble Nitrogen	Alcohol-Soluble Nitrogen
	%	%	%	%	%	%
Guatemalan Cuyuta	8.0	16.8	10.8	30.8	53.8	4.6
U.S. 4251	8.2	16.1	0.9	39.4	54.0	5.7
San Sebastian (SSD) (Guat.)	8.6	17.4	0.0	40.8	49.0	10.2
Guatemalan 142–48	11.4	18.4	6.6	37.2	50.8	5.4
U.S. High Oil HO	12.4	17.4	5.1	36.0	53.4	5.5
U.S. High Protein H5	14.0	20.4	4.7	36.0	53.3	6.0

varieties. Insoluble nitrogen varied from 0 to 10.8%. Acid-soluble nitrogen varied from 30.8% in Guatemalan Cuyuta to 40.8% in Guatemalan San Sabastian (SSD), and alkali-soluble nitrogen varied from 49% in San Sebastian (SSD) to 54% in U.S. 4251. The alcohol-soluble nitrogen is the minor fraction in germ, varying from 4.6 to 10.2%. No relationship is apparent between the total protein of the grain and the distribution of nitrogen in the germ. With the exception of Guatemalan Cuyuta, nitrogen distribution appears to be about the same for all varieties tested.

Lysine Content of Mixed Fractions. Because of the limited amounts of the separated germs, endosperms, and soluble fractions available, similar anatomical and chemical fractions were pooled without reference to amount, and analyzed for total nitrogen and lysine. The data are shown in Table IV. The mixed endosperm sample contained

TABLE IV

LYSINE CONTENT OF COMPOSITE FRACTIONS

FRACTION	SOURCE (CORN NO.)[a]	NITROGEN[b]	LYSINE[c]
		%	%
Endosperm	1,2,3,7,8,9,10	1.78	1.43
Endosperm extract	1,4	10.52	1.34
Endosperm acid-soluble	1,2,3,4,7,8,9,10	6.78	2.90
Endosperm alkali-soluble	1,2,7,8,9,10	14.52	2.16
Endosperm alcohol-soluble	1,2,3,7,8,9,10	13.53	0.08
Germ	1,2,3,8,9,10	4.32	5.51
Germ extract	1,4,10	11.32	5.33
Germ acid-soluble	1,2,3,8,9,10	7.84	6.43
Germ alkali-soluble	1,2,3,8,9,10	13.09	5.80
Germ alcohol-soluble	1,2,3,8,9,10	9.89	1.05

[a] Corn numbers listed in Table I.
[b] Dry basis.
[c] Percent of crude protein (N × 6.25).

less than one-half the level of total nitrogen and about one-fourth the level of lysine present in the mixed germ sample. Among the endo-

sperm fractions, the mixed acid-soluble fraction contained the highest level of lysine (2.9%), the mixed alkali-soluble (glutelin) fraction was somewhat lower (2.16%), and the mixed alcohol-soluble fraction was very low (0.08%). The relatively low nitrogen content of the mixed acid-soluble endosperm fraction (6.78%) was probably due to the presence of a starch component (12).

The mixed acid- and mixed alkali-soluble fractions of the germ contained high levels of lysine (6.4 and 5.8% respectively), whereas the mixed alcohol-soluble fraction was relatively low in lysine (1.0%). Since the various fractions were pooled without reference to amount of individual varieties, the data are included for their informative rather than quantitative value.

Amino Acid Composition of Whole Grain. For purposes of comparison, four U.S. and four Guatemalan corn selections and teosinte were analyzed for 18 amino acids and total nitrogen. These data are presented in Table V. No major differences in amino acid composition

TABLE V

AMINO ACID CONTENT OF CORN AND TEOSINTE

AMINO ACID	CUYUTA	4251	SSD	TGY	142-48	HO	H5	HP	TEOSINTE
	%	%	%	%	%	%	%	%	%
(Nitrogen)	1.28	1.31	1.37	1.57	1.83	1.99	2.24	2.91	3.81[b]
Alanine	7.5[a]	8.9	12.5	10.3	8.6	6.4	10.8	9.9	8.5
Arginine	3.5	3.9	3.6	4.1	2.9	4.6	3.6	3.9	2.9
Aspartic acid	6.5	6.2	5.8	6.1	6.0	6.0	6.8	6.1	5.3
Cystine	1.4	1.6	1.4	1.4	1.6	1.5	1.2	1.4	1.1
Glutamic acid	15.4	14.6	14.0	13.5	15.0	13.9	12.4	12.8	19.9
Glycine	3.1	3.3	2.8	2.9	2.6	3.4	2.6	2.8	2.2
Histidine	2.1	2.8	2.0	2.2	2.1	2.3	2.0	2.2	1.9
Isoleucine	2.6	3.3	2.7	3.4	3.0	3.5	3.7	4.0	4.7
Leucine	10.5	12.2	12.0	12.2	13.4	7.8	13.6	15.2	16.8
Lysine	2.8	2.9	2.1	2.6	2.3	3.2	2.1	2.0	1.3
Methionine	1.3	1.6	1.2	1.0	1.0	1.2	1.7	1.1	1.2
Phenylalanine	4.4	4.5	4.6	4.4	5.4	2.9	5.3	5.7	5.7
Proline	8.1	10.3	7.4	6.6	9.6	9.3	8.3	6.8	9.6
Serine	4.5	4.6	4.2	4.6	4.5	4.8	5.0	5.5	5.2
Threonine	3.1	3.3	2.9	3.1	3.0	3.2	3.1	3.3	3.0
Tryptophan	0.63	0.49	0.47	0.51	0.44	0.56	0.43	0.44	0.38
Tyrosine	2.9	3.4	3.0	3.0	3.3	3.5	3.6	4.1	4.4
Valine	4.1	4.6	4.1	4.3	4.0	2.1	4.3	4.6	4.8

[a] Percent of crude protein ($N \times 6.25$).
[b] Dehulled.

were observed between the U.S. corn selections, the Guatemalan corn selections, and teosinte. Some differences in the amino acid composition of the low and high protein corns of each country are apparent (for example, leucine, Table V). Differences of this nature have been observed previously by Sauberlich *et al.* (14) and other workers, in U.S. low- and high-protein corns.

Weight and Nitrogen Distribution of Anatomical Fractions. Table VI shows the percent of the weight and nitrogen contributed by the

TABLE VI

DISTRIBUTION OF WEIGHT AND NITROGEN AMONG ANATOMICAL FRACTIONS

CORN SAMPLE	WEIGHT OF 20 SEEDS	WEIGHT DISTRIBUTION			TOTAL N	NITROGEN DISTRIBUTION		
		Seed Coat[a]	Endosperm	Germ		Seed Coat	Endosperm	Germ
	g	%	%	%	%	%	%	%
U.S. 4251	5.62	6.3	86.3	7.4	1.31	3.3	81.2	15.5
U.S. High Oil (HO)	5.72	6.4	71.2	22.4	1.99	2.4	68.4	29.2
U.S. High Protein (H5)	4.32	6.9	82.7	10.4	2.24	2.2	83.2	14.6
U.S. High Protein (HP)	4.97	7.4	78.9	13.7	2.91	1.8	83.0	15.2
U.S. Normal-*Sh*[1]	4.38	6.7	79.6	13.7	2.14	2.7	78.2	19.1
U.S. Mutant-*sh*[1]	2.50	10.7	70.6	18.7	2.21	6.1	64.6	29.3
Tiguisate (TGY) (Guat.)	5.43	6.5	81.3	12.2	1.57	3.1	75.4	21.4
San Sebastian (SSD) (Guat.)	8.24	4.9	83.9	11.2	1.37	2.8	75.2	22.0
Guatemalan 142–48	6.91	6.9	82.1	11.0	1.83	2.6	81.0	16.4
Guatemalan Cuyuta	5.95	5.7	82.5	11.8	1.28	2.9	72.4	24.7
Guatemalan teosinte	1.56	55.6[b]	44.4[c]	...	1.81[d]	8.2	91.8[c]	...

[a] Pericarp plus tip cap.
[b] Includes the seed coat (1.3%) and the hulls (54.3%).
[c] Includes the germ.
[d] The hulls contained 0.26% nitrogen. Dehulled teosinte contained 3.81% nitrogen.

seed coat (pericarp plus tip cap) endosperm and germ in ten of the corn varieties studied, and in teosinte. The percent of the total weight contributed by the pericarp plus tip cap varied from 4.9 in Guatemalan San Sebastian (SSD) to 7.4 in U.S. High Protein (HP); the percent contributed by the endosperm varied from 71.2 in U.S. High Oil (HO) to 86.3 in U.S. 4251; the percent contributed by the germ varied from 7.4 in U. S. 4251 to 22.4 in U.S. High Oil (HO). The percent of the total nitrogen of the grain contributed by the pericarp plus tip cap varied from 1.8 in U.S. High Protein (HO) to 3.3 in U.S. 4251; the percent contributed by the endosperm varied from 68.4 in U.S. High Oil (HO) to 83.2 in U.S. High Protein (H5); the percent contributed by the germ varied from 14.6 in U.S. High Protein (H5) to 29.2 in U.S. High Oil (HO).

Use of Fractionation Method in Corn Breeding. The quality of corn protein assumes special importance in certain areas of the United States where corn is used in animal feeds; thus, in the Midwest, swine and poultry feeds contain 50–70% of this grain. Protein quality is even more crucial in areas of the world where corn serves as a major source of human food (1, 2, 4).

If the content of lysine alone is considered as the criterion for protein quality, our data show that the percent of lysine in the whole kernel can be increased by (a) breeding for larger germ size (see HO, Tables V and VI, and germ, Table IV) and (b) by increasing the

relative percentages of the acid-soluble and alkali-soluble fractions of the endosperm (see 4251 and Cuyuta, Tables II and V, and endosperm fractions, Table IV). This conclusion was also reached by Frey and co-workers (6, 7), using different fractionation techniques.

In addition to the need for corn varieties containing better protein quality, it is possible that expanding uses for regenerated zein fibers such as Vicara may create a demand for a small-germ, high-protein corn containing a high percentage of alcohol-soluble nitrogen (see U.S. High Protein H5 and HP, Table II). The copper extraction-fractionation method should prove useful to the corn breeder in the development of both of these desirable food and fiber types of corn.

Acknowledgments

We wish to thank Dr. D. L. Richardson and Dr. A. M. Brunson, Department of Botany and Plant Pathology, for the U.S. shrunken-floury (*sh''*) samples and the U.S. high protein samples, respectively. We are also indebted to Mr. C. Bressani, Guatemala, C.A., for the teosinte and teosinte-corn cross samples, and to INCAP, Guatemala, C.A., for the four samples of Guatemalan corn varieties.

Literature Cited

1. Aguirre, F., Bressani, R., and Scrimshaw, N. S. The nutritive value of Central American corns. III. Tryptophan, niacin, thiamine, and riboflavin content of twenty-three varieties in Guatemala. Food Research **18:** 273–279 (1953).
2. Aguirre, F., Robles, C. E., and Scrimshaw, N. S. The nutritive value of Central American corns. II. Lysine and methionine content of twenty-three varieties in Guatemala. Food Research **18:** 268–272 (1953).
3. Association of Official Agricultural Chemists. Official and tentative methods of analysis (6th ed.). The Association: Washington, D.C. (1945).
4. Bressani, R., Arroyave, G., and Scrimshaw, N. S. The nutritive value of Central American corns. I. Nitrogen, ether extract, crude fiber, and minerals of twenty-four varieties in Guatemala. Food Research **18:** 261–267 (1953).
5. Bressani, R., Campos, A. A., Squibb, R. L., and Scrimshaw, N. S. The nutritive value of Central American corns. IV. The carotene content of thirty-two selections of Guatemalan corn. Food Research **18:** 618–624 (1953).
6. Frey, K. J. The interrelationships of proteins and amino acids in corn. Cereal Chem. **28:** 123–132 (1951).
7. Frey, K. J., Brimhall, B., and Sprague, G. F. The effects of selection upon protein quality in the corn kernel. Agron. J. **41:** 399–403 (1949).
8. Hansen, D. W., Brimhall, B., and Sprague, G. F. Relationship of zein to the total protein in corn. Cereal Chem. **23:** 329–335 (1946).
9. Hamilton, T. S., Hamilton, Barbara C., Johnson, B. C., and Mitchell, H. H. The dependence of the physical and chemical composition of the corn kernel on soil fertility and cropping system. Cereal Chem. **28:** 163–176 (1951).
10. Lloyd, N. E., and Mertz, E. T. Studies on corn proteins. III. The glutelins of corn. Cereal Chem. (in press).
11. Mertz, E. T., and Bressani, R. Studies on corn proteins. I. A new method of extraction. Cereal Chem. **34:** 63–69 (1957.)
12. Mertz, E. T., Lloyd, N. E., and Bressani, R. Studies on corn proteins. II. Electrophoretic analysis of germ and endosperm extracts. Cereal Chem. (in press).
13. Richardson, D. L. Shrunken-floury, a gene affecting protein synthesis. Maize Genetics Cooperation News Letter **29:** 45–46 (1955).
14. Sauberlich, H. E., Chang, W., and Salmon, W. D. The amino acid and protein content of corn as related to variety and nitrogen fertilization. J. Nutrition **51:** 241–250 (1953).

15. SCHNEIDER, E. D., EARLY, E. B., and DETURK, E. E. Nitrogen fractions of the component parts of the corn kernel as affected by selection and soil nitrogen. Agron. J. **44:** 161–169 (1952).
16. STEELE, B. F., SAUBERLICH, H. E., REYNOLDS, M. S., and BAUMANN, C. A. Media for *Leuconostoc mesenteroides* P-60 and *Leuconostoc citrovorum* 8081. J. Biol. Chem. **177:** 533–544 (1949).
17. WINTON, A. L., and WINTON, K. B .The structure and composition of foods, Vol. I, pp. 98–100. Wiley: New York (1932).

THE RATIO OF ALCOHOL-SOLUBLE TO TOTAL NITROGEN IN DEVELOPING OAT SEEDS[1]

S. C. WIGGANS AND K. J. FREY[2]

ABSTRACT

Oat seeds harvested at 2- or 3-day intervals during the period from 4 days after anthesis until maturity varied in total and alcohol-soluble nitrogen percentages. In 1955 the total nitrogen remained nearly constant but in 1956 it increased markedly as the seeds developed. In both years the alcohol-soluble nitrogen decreased somewhat during the first 8 to 14 days after anthesis and then later returned to the original percentages at maturity. The ratio of alcohol-soluble to total nitrogen decreased for the first 16 to 20 days following anthesis.

The grain of cereals generally contains a considerable quantity of alcohol-soluble nitrogen (2, 3, 4). In corn the proportion of alcohol-soluble to total nitrogen becomes greater as the nitrogen percentage in the corn grain increases (5), whereas in oats this ratio is relatively constant over a wide range of nitrogen percentages (3). However, during the maturation of corn grain, regardless of the inherent nitrogen level, the ratio of zein to total nitrogen tends to increase[3], while the total nitrogen percentage decreases consistently.

The objectives of the present study were twofold: (a) to determine the total nitrogen percentages in developing oat seeds, and (b) to find the relationship between the total and alcohol-soluble nitrogen in the same material. For all of the chemical analyses no attempt was made to separate the hulls and caryopses of the oat seeds since oats are fed to livestock in this way.

[1] Manuscript received June 10, 1957. Journal Paper No. J-3220 of the Iowa Agricultural Experiment Station, Ames, Iowa. Projects 1139 and 1176. In cooperation with Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

[2] Assistant Professor of Farm Crops and of Botany and Professor of Farm Crops, respectively, and collaborators, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

[3] Unpublished data from Dr. G. F. Sprague.

Materials and Methods

The seed samples were collected from the oat varieties Sauk and Mo. 0-205 grown at Ames, Iowa, in 1955 and 1956. The field planting in each case consisted of a series of rows each 8 ft. long and spaced 1 ft. apart. The planted area was divided into three parts to represent replications, and at a given date a number of randomly selected rows were harvested from each replication.

A seed sample was collected by stripping the spikelets from the upper halves of the oat heads. (Using only the upper halves of the panicles ensured better uniformity of maturity among the spikelets, since anthesis begins with the uppermost spikelet and takes from 3 to 5 days to complete on an individual panicle.) The seed samples were then placed on paper towels and dried at room temperature to a moisture content safe for storage.

The collection of seed lots was started 4 days after anthesis and samples were taken subsequently at 2- or 3-day intervals until the moisture content of the seeds on the plants was reduced to 13%. The number of samples collected in the 2 years varied because the rates of maturation were so different. In 1955, the season was cool and humid which resulted in a long grain-filling period, whereas in 1956 the season was hot and dry resulting in a short grain-maturation interval.

About 60 days after the last harvest the seed samples were threshed free of glumes and ground in a Wiley mill to pass through a 20-mesh screen. Total nitrogen was determined by the method described by the Association of Official Agricultural Chemists (1) using 0.5-g. samples. The procedure used to determine the alcohol-soluble nitrogen has been described by Frey (3).

Results and Discussion

The percentages of total nitrogen in the oat seeds are shown graphically in Fig. 1. In 1955 there was little change in the percent of total nitrogen throughout the period from 4 days after anthesis to maturity, although there was a slight tendency for total nitrogen to increase with advancing maturity. In 1956, however, there was a very sharp increase in the percent of total nitrogen until approximately 16 days after anthesis, after which the total nitrogen percentage remained relatively constant. These data indicate that the deposition of total nitrogen in the oat grain occurred at a rate at least as rapid as that of the other grain components. In 1956, the rate of nitrogen deposition was relatively greater than that of the other components. This may have resulted from the crop's ripening more rapidly than normally because of excessive heat and drought.

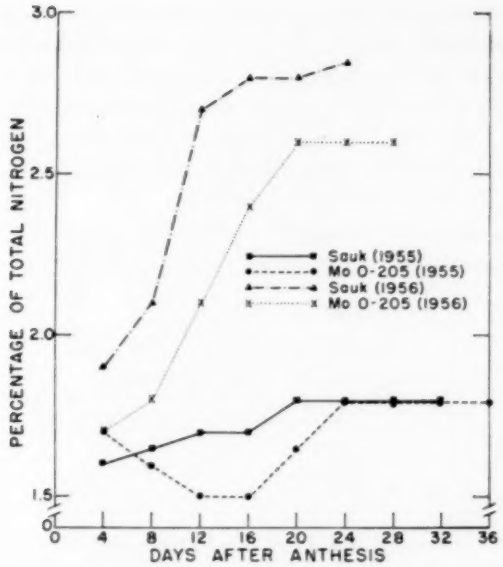

Fig. 1. The percent of total nitrogen at varying days after anthesis for two oat varieties in 1955 and 1956.

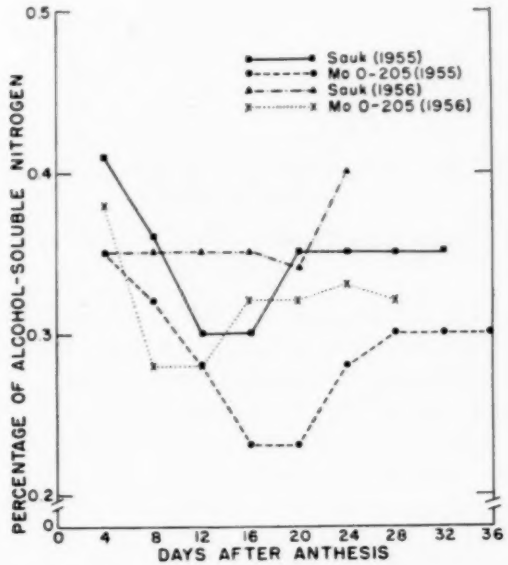

Fig. 2. The percent of alcohol-soluble nitrogen at varying days after anthesis for two oat varieties in 1955 and 1956.

The percentages of alcohol-soluble nitrogen in the developing oat seeds are presented in Fig. 2. The changes in the percentage of alcohol-soluble nitrogen during the seed maturation period were small. In three of the four cases there was a slight decrease until 8 to 14 days after anthesis, following which there was a tendency to return to the original level. In general, Sauk contained a higher percent of alcohol-soluble nitrogen than did Mo. 0-205.

The ratios of alcohol-soluble to total nitrogen are given in Fig. 3. There was a consistent decrease in this ratio from the first sampling

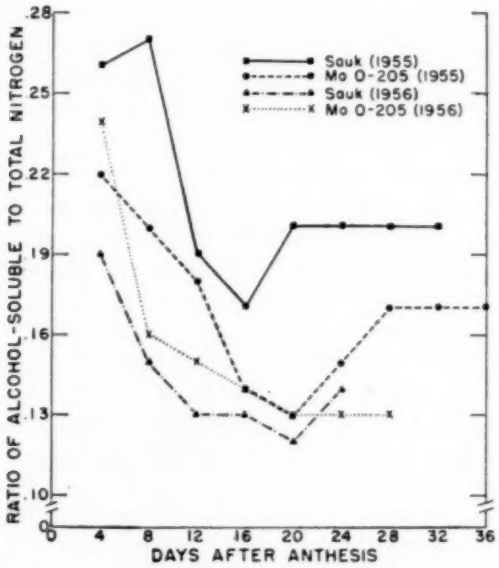

Fig. 3. The ratio of alcohol-soluble to total nitrogen at varying days after anthesis for two oat varieties in 1955 and 1956.

date until from 16 to 20 days after anthesis. The drop in the ratio during the initial development of the oat seeds amounted to about 25 to 35%. The relationship between alcohol-soluble and total nitrogen displayed by the developing oat seeds herein, is somewhat contrary to that found for corn (see footnote 3). The ratio of alcohol-soluble to total nitrogen in corn increases with advancing maturity, whereas in oats it has a relatively long period of decrease before it increases again.

It appears from the data reported herein and a paper by Frey (3) that the alcohol-soluble/total nitrogen ratio behaves differently in corn and oats. In corn the ratio tends to increase with advancing maturation

of the corn kernels and with an increasing nitrogen percentage in the grain. In contrast, the ratio in oats decreases in the initial stages of seed development and is relatively constant irrespective of the total nitrogen characteristic of the variety. The effects of the hull on the oat grain on these ratios are unknown at present.

Literature Cited

1. Association of Official Agricultural Chemists. Official methods of analysis (7th ed.). The Association: Washington, D. C. (1950).
2. Frey, K. J. The interrelationships of proteins and amino acids in corn. Cereal Chem. **28:** 123–132 (1951).
3. Frey, K. J. The relation between alcohol-soluble and total nitrogen contents of oats. Cereal Chem. **28:** 506–509 (1951).
4. Hanson, D. W., Brimhall, B., and Sprague, G. F. Relationship of zein to the total protein in corn. Cereal Chem. **23:** 329–335 (1946).
5. Showalter, M. F., and Carr, R. H. Characteristic proteins in high- and low-protein corn. J. Am. Chem. Soc. **44:** 2019–2023 (1922).

COMMUNICATION TO THE EDITOR

Effects of Water-Saturated n-Butanol on Gluten

DEAR SIR:

Mecham and Mohammad reported (Cereal Chem. **32:** 405–415, 1955) that gluten from flour extracted with water-saturated n-butanol is recoverable and of fair quality. Since this solvent removed a larger quantity of lipid substances than did any other used, such extraction appeared to offer possibilities of improved preparation of gluten for use in fundamental physical chemical studies.

The effects of extraction of lipids with water-saturated n-butanol were thoroughly investigated. The amounts of lipids extracted from three different flours agreed reasonably well with those reported by Mecham and Mohammad (0.90% lipids extracted by ether, 1.44% by n-butanol). Gluten was readily recovered from all extracted flours by use of mechanical washing. The gluten recovered from butanol-extracted flour was granular in nature and, while it could be worked into a somewhat coherent mass, this mass was very short and of poor quality. The quality of this gluten was definitely superior to that of gluten from ethanol-extracted flour, but definitely inferior to that of the gluten from nonextracted or ether-extracted flour.

The washed glutens were dispersed in 8% sodium salicylate solutions. There was no residue of undispersed gluten from ether-extracted flours but there was always a small, undispersed, granular residue of gluten from butanol-extracted flour. All dispersions were readily clarified at 20,000 × G and the amount of sediment was always greater from the butanol-extracted samples.

The amount of dispersed protein was determined by the Kjeldahl method and the results consistently showed that butanol extraction resulted in a loss of about 6% in solubility as compared with ether extraction. The clarified dispersions were subjected to sedimentation analysis in a Model E Spinco Ultracentrifuge at 250,000 gravities. The amount of molecularly dispersed gluten (see McCalla and Gralén, Can. J. Research **C20:** 130–159, 1942) was about 8% less for the gluten from butanol-extracted flour. These figures are considerably lower than the 15% reported by Mecham and Mohammad.

Despite the smaller loss in dispersibility, consistent differences were obtained in the amide nitrogen content of both dispersed gluten and nondispersed residues. The amide values were invariably higher when dispersibility was reduced, and are therefore in complete agreement with all earlier results obtained in this laboratory.

The results obtained in this study confirm the claim that butanol-extracted flours yield recoverable gluten of better quality than that recovered from ethanol-extracted flours. Nevertheless, the colloidal properties of the gluten were seriously altered. In general, it is concluded that the method is not satisfactory for use in the preparation of gluten for fundamental studies.

December 2, 1957

M. B. BHATTI
Chemical Section, Punjab Agricultural College, Lyallpur, W. Pakistan
(Formerly Colombo Plan Fellow at University of Alberta)
and
A. G. MCCALLA
Dean, Faculty of Graduate Studies, University of Alberta, Edmonton

COMMUNICATION TO THE EDITOR

Use of Weston Rolls for Preparing Small Samples of Flour

DEAR SIR:

Attention should probably be called to the fact that the use of the rolls of the Weston Moisture Meter for preparing small samples of flour as proposed by Schlesinger (Cereal Chemistry **34:** 433–436; 1957) is not new. The use of the Weston rolls in a very similar manner for preparing flour for the sedimentation test was previously described by Pinckney *et al.* (Cereal Chemistry **34:** 16–25; 1957) and is included in the sedimentation test procedure described in *Cereal Laboratory Methods,* 6th edition.

Schlesinger's successful use of this flour in farinography points to the possible wider application of this simple device in laboratories where experimental flour milling equipment is not available. The U. S. Department of Agriculture is considering the project of developing a simple and inexpensive mill for preparing small samples of flour of reasonably low ash content for the sedimentation test and other similar uses.

February 14, 1958

LAWRENCE ZELENY
Chief, Standardization and Testing Branch
Grain Division
Agricultural Marketing Service
U. S. Dept. of Agriculture
Washington 25, D. C.

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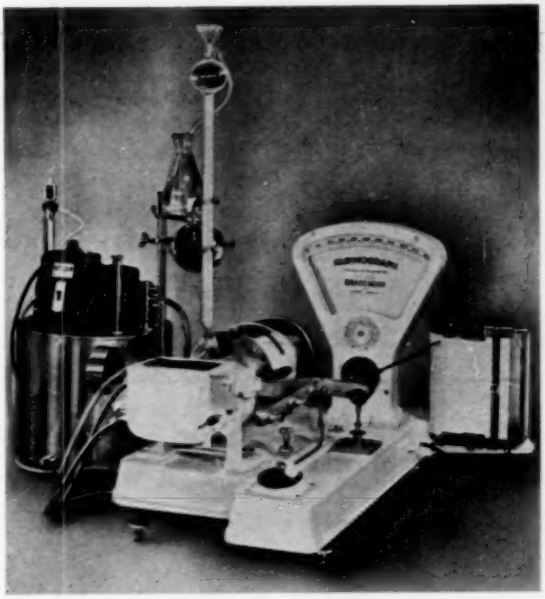

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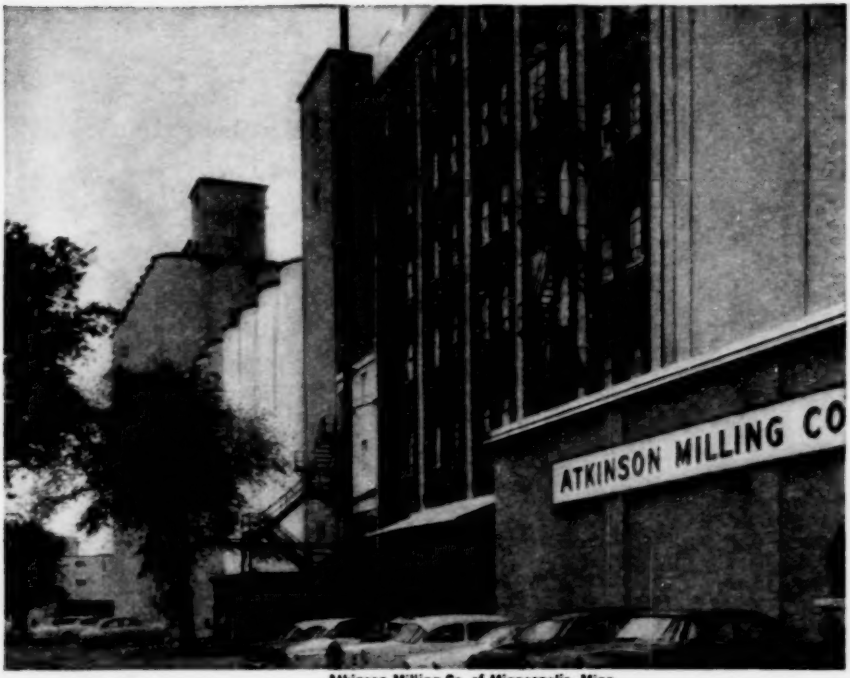

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